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(54) Title: NOVEL HUMAN G-PROTEIN COUPLED RECEPTOR

(57) Abstract: The present invention relates to novel identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to the G-protein coupled receptor family, referred to as IGS4-family. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides, to a vector containing said polynucleotides, a host cell containing such vector and transgenic animals where the IGS4-gene is either overexpressed, misexpressed, underexpressed or suppressed (knock-out animals). The invention further relates to a method for screening compounds capable to act as an agonist or an antagonist of said G-protein coupled receptor family IGS4 and the use of IGS4 polypeptides and polynucleotides and agonists or antagonists to the IGS4 receptor family in the treatment of PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension - e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease - e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers - e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, among others and diagnostic assays for such conditions. Preferred uses of the invention relate to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system. The invention also relates to the identification of the cognate ligand of the IGS4 polypeptides of the invention. High affinity binding to said IGS4 polypeptides is found for the neuropeptides known as neuromedin U.

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Novel human G-protein coupled receptor

Description

The present invention relates to novel identified polynucleotides, polypeptides encoded by them and to the use of such polynucleotides and polypeptides, and to their production. More particularly, the polynucleotides and polypeptides of the present invention relate to a G-protein coupled receptor (GPCR), hereinafter referred to as IGS4. IGS4 exists in two polymorphic forms, hereinafter referred to as IGS4A and IGS4B. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides, to a vector containing said polynucleotides, a host cell containing such vector and transgenic animals where the IGS4-gene is either overexpressed, misexpressed, underexpressed and/or suppressed (knock-out animals). The invention further relates to a method for screening compounds capable to act as an agonist or an antagonist of said G-protein coupled receptor IGS4, and to the cognate ligand of IGS4.

BACKGROUND OF THE INVENTION

It is well established that many medically significant biological processes are mediated by proteins participating in signal transduction pathways that involve G-proteins and/or second messengers; e.g., cAMP (Lefkowitz, Nature, 1991, 351:353-354). Herein these proteins are referred to as proteins participating in pathways with G-proteins. Some examples of these proteins include the GPC receptors, such as those for adrenergic agents and dopamine (Kobilka, B.K., et al., Proc. Natl. Acad. Sci., USA, 1987, 84:46-50; Kobilka, B.K., et al., Science, 1987, 238:650-656; Bunzow, J.R., et al., Nature, 1988, 336:783-787), G-proteins themselves, effector proteins, e.g., phospholipase C, adenylate cyclase, and phosphodiesterase, and actuator proteins, e.g., protein kinase A and protein kinase C (Simon, M.I., et al., Science, 1991, 252:802-8).

For example, in one form of signal transduction, upon hormone binding to a GPCR the receptor interacts with the heterotrimeric G-protein and induces the dissociation of GDP from the guanine nucleotide-binding site. At normal cellular concentrations of guanine nucleotides, GTP fills the site immediately. Binding of GTP to the α subunit of the G-protein causes the dissociation of the G-protein from the receptor and the dissociation of the G-protein into α and $\beta\gamma$ subunits. The GTP-carrying form then binds to activated adenylate cyclase. Hydrolysis of GTP to GDP, catalyzed by the G-protein itself (α subunit possesses an intrinsic GTPase activity), returns the G-protein to its basal, inactive form. The GTPase activity of the α subunit is, in essence, an internal clock that controls an on/off switch. The GDP bound form of the α subunit has high affinity for $\beta\gamma$ and subsequent reassociation of αGDP with $\beta\gamma$ returns the system to the basal state. Thus the G-protein serves a dual role, as an intermediate that relays the signal from receptor to effector (in this example adenylate cyclase), and as a clock that controls the duration of the signal.

The membrane bound superfamily of G-protein coupled receptors has been characterized as having seven putative transmembrane domains. The domains are believed to represent transmembrane α-helices connected by extracellular or cytoplasmic loops. G-protein coupled receptors include a wide range of biologically active receptors, such as hormone, viral, growth factor and neuroreceptors.

The G-protein coupled receptor family includes dopamine receptors which bind to neuroleptic drugs used for treating CNS disorders. Other examples of members of this family include, but are not limited to calcitonin, adrenergic, neuropeptideY, somastotatin, neurotensin, neurokinin, capsaicin, VIP, CGRP, CRF, CCK, bradykinin, galanin, motilin, nociceptin, endothelin, cAMP, adenosine, muscarinic, acetylcholine, serotonin, histamine, thrombin, kinin, follicle stimulating hormone, opsin, endothelial differentiation gene-1, rhodopsin, odorant, and cytomegalovirus receptors.

Most G-protein coupled receptors have single conserved cysteine residues in each of the first two extracellular loops which form disulfide bonds that are believed to stabilize functional protein structures. The 7 transmembrane regions are designated as TM1, TM2, TM3, TM4, TM5, TM6 and TM7. The cytoplasmic loop which connects TM5 and TM6 may be a major component of the G-protein binding domain.

Most G-protein coupled receptors contain potential phosphorylation sites within the third cytoplasmic loop and/or the carboxy terminus. For several G-protein coupled receptors, such as the β-adrenoreceptor, phosphorylation by protein kinase A and/or specific receptor kinases mediates receptor desensitization.

Recently, it was discovered that certain GPCRs, like the calcitonin-receptor like receptor, might interact with small single pass membrane proteins called receptor activity modifying proteins (RAMP's). This interaction of the GPCR with a certain RAMP is determining which natural ligands have relevant affinity for the GPCR-RAMP combination and regulate the functional signaling activity of the complex (McLathie, L.M. et al., Nature (1998) 393:333-339).

For some receptors, the ligand binding sites of G-protein coupled receptors are believed to comprise hydrophilic sockets formed by several G-protein coupled receptor transmembrane domains, said sockets being surrounded by hydrophobic residues of the G-protein coupled receptors. The hydrophilic side of each G-protein coupled receptor transmembrane helix is postulated to face inward and form a polar ligand-binding site. TM3 has been implicated in several G-protein coupled receptors as having a ligand-binding site, such as the TM3 aspartate residue. TM5 serines, a TM6 asparagine and TM6 or TM7 phenylalanines or tyrosines are also implicated in ligand binding.

G-protein coupled receptors can be intracellularly coupled by heterotrimeric G-proteins to various intracellular enzymes, ion channels and transporters (see, Johnson et al., Endoc. Rev., 1989, 10:317-331). Different G-protein α-subunits preferentially stimulate particular effectors to modulate various biological functions in a cell. Phosphorylation of cytoplasmic residues of G-protein coupled receptors has been identified as an important mechanism for the regulation of G-protein coupling of some G-protein coupled receptors. G-protein coupled receptors are found in numerous sites within a mammalian host.

Receptors - primarily the GPCR class - have led to more than half of the currently known drugs (Drews, Nature Biotechnology, 1996, 14: 1516). This indicates that these receptors have an established, proven history as therapeutic targets. Clearly there is a need for identification and characterization of further receptors which can play a role in preventing, ameliorating or correcting dysfunctions or diseases, including, but not limited to PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders.

SUMMARY OF THE INVENTION

In one aspect, the invention relates to IGS4 polypeptides (including the IGS4A and IGS4B polypeptide polymorphs), polynucleotides and recombinant materials and methods for their production. Another aspect of the invention relates to methods for using such IGS4 polypeptides, polynucleotides and recombinant materials. Such uses include, but are not limited to, use as a therapeutic target and for the treatment of PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal

anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, among others. Preferred uses of the invention relate to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

In still another aspect, the invention relates to methods to identify agonists and antagonists using the materials provided by the invention, and treating conditions associated with IGS4 imbalance with the identified compounds. Yet another aspect of the invention relates to diagnostic assays for detecting diseases associated with inappropriate IGS4 activity or levels. A further aspect of the invention relates to animal-based systems which act as models for disorders arising from aberrant expression or activity of IGS4. Preferred agonists or antagonists identified according to the present invention are those which are suited for the treatment of disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

The invention also relates to the identification of the cognate ligand of the IGS4 polypeptides of the invention. High affinity binding to said IGS4 polypeptides is found for the neuropeptides known as neuromedin U.

Table 1: IGS4A-DNA of SEQ ID NO: 1 and SEQ ID NO: 3

5'-
GGCTCAGCTTGAAACAGAGCCTCGTACCAGGGGAGGCTCAGGCCTTGGATTTTAATGTCA
GGGATGGAAAAACTTCAGAATGCTTCCTGGATCTACCAGCAGAAACTAGAAGATCCATTC
CAGAAACACCTGAACAGCACCGAGGAGTATCTGGCCTTCCTCTGCGGACCTCGGCGCAGC
CACTTCTTCCTCCCCGTGTCTGTGGTGTATGTGCCAATTTTTGTGGTGGGGGTCATTGGC
AATGTCCTGGTGTGCCTGGTGATTCTGCAGCACCAGGCTATGAAGACGCCCACCAACTAC
TACCTCTTCAGCCTGGCGGTCTCTGACCTCCTGGTCCTGCTCCTTGGAATGCCCCTGGAG
GTCTATGAGATGTGGCGCAACTACCCTTTCTTGTTCGGGCCCGTGGGCTGCTACTTCAAG
ACGGCCCTCTTTGAGACCGTGTGCTTCGCCTCCATCCTCAGCATCACCACCGTCAGCGTG
GAGCGCTACGTGGCCATCCTACACCCGTTCCGCGCCAAACTGCAGAGCACCCGGCGCCGG
GCCCTCAGGATCCTCGGCATCGTCTGGGGCTTCTCCGTGCTCTTCTCCCTGCCCAACACC
AGCATCCATGGCATCAAGTTCCACTACTTCCCCAATGGGTCCCTGGTCCCAGGTTCGGCC
ACCTGTACGGTCATCAAGCCCATGTGGATCTACAATTTCATCATCCAGGTCACCTCCTTC
CTATTCTACCTCCTCCCCATGACTGTCATCAGTGTCCTCTACTACCTCATGGCACTCAGA
CTAAAGAAAGACAAATCTCTTGAGGCAGATGAAGGGAATGCAAATATTCAAAGACCCTGC
AGAAAATCAGTCAACAAGATGCTGTTTGTCTTGGTCTTAGTGTTTGCTATCTGTTGGGCC
CCGTTCCACATTGACCGACTCTTCTTCAGCTTTGTGGAGGAGTGGAGTGAATCCCTGGCT
GCTGTGTTCAACCTCGTCCATGTGGTGTCAGGTGTCTTCTTCTACCTGAGCTCAGCTGTC
AACCCCATTATCTATAACCTACTGTCTCGCCGCTTCCAGGCAGCATTCCAGAATGTGATC
TCTTCTTTCCACAAACAGTGGCACTCCCAGCATGACCCACAGTTGCCACCTGCCCAGCGG
AACATCTTCCTGACAGAATGCCACTTTGTGGAGCTGACCGAAGATATAGGTCCCCAATTC
CCATGTCAGTCATCCATGCACAACTCTCACCTCCCAACAGCCCTCTCTAGTGAACAGATG
TCAAGAACAAACTATCAAAGCTTCCACTTTAACAAAACCTGAATTCTTTCAGAGCTGACT
CTCCTCTATGCCTCAAAACTTCAGAGAGGAACATCCCATAATGTATGCCTTCTCATATGA
TATTAGAGAGGTAGAATGGCTCTTACAACTCATGTACCCATTGCTAGTTTTTTTTTTTTA
ATAAACGTGAAAACTGAGAGTTAGATCTGGTTTCAAAACCCAAGACTGCCTGATTTTTAG
TTATCTTTCCACTATCCTAACTGCCTCATGCCCCTTCACTAGTTCATGCCAAGAACGTGA
CTGGAAAGGCATGGCACCTATACCTTGATTAATTTCCATTAATGGAAATGGTTCGTCCTG
AGTCATCTACGTTCCGAGTCAGGCTGTCACTCCTACTA-3'

Table 2: IGS4B-DNA of SEQ ID NO: 5 and SEQ ID NO: 7

5'-
GGCTCAGCTTGAAACAGAGCCTCGTACCAGGGGAGGCTCAGGCCTTGGATTTTAATGTCA
GGGATGGAAAAACTTCAGAATGCTTCCTGGATCTACCAGCAGAAACTAGAAGATCCATTC
CAGAAACACCTGAACAGCACCGAGGAGTATCTGGCCTTCCTCTGCGGACCTCGGCGCAGC
CACTTCTTCCTCCCCGTGTCTGTGGTGTATGTGCCAATTTTTGTGGTGGGGGTCATTGGC
AATGTCCTGGTGTGCCTGGTGATTCTGCAGCACCAGGCTATGAAGACGCCCACCAACTAC
TACCTCTTCAGCCTGGCGGTCTCTGACCTCCTGGTCCTGCTCCTTGGAATGCCCCTGGAG
GTCTATGAGATGTGGCGCAACTACCCTTTCTTGTTCGGGCCCGTGGGCTGCTACTTCAAG
ACGGCCCTCTTTGAGACCGTGTGCTTCGCCTCCATCCTCAGCATCACCACCGTCAGCGTG
GAGCGCTACGTGGCCATCCTACACCCGTTCCGCGCCAAACTGCAGAGCACCCGGCGCCGG
GCCCTCAGGATCCTCGGCATCGTCTGGGGCTTCTCCGTGCTCTTCTCCCTGCCCAACACC
AGCATCCATGGCATCAAGTTCCACTACTTCCCCAATGGGTCCCTGGTCCCAGGTTCGGCC
ACCTGTACGGTCATCAAGCCCATGTGGATCTACAATTTCATCATCCAGGTCACCTCCTTC
CTATTCTACCTCCTCCCCATGACTGTCATCAGTGTCCTCTACTACCTCATGGCACTCAGA
CTAAAGAAAGACAAATCTCTTGAGGCAGATGAAGGGAATGCAAATATTCAAAGACCCTGC
AGAAAATCAGTCAACAAGATGCTGTTTGTCTTGGTCTTAGTGTTTGCTATCTGTTGGGCC
CCGTTCCACATTGACCGACTCTTCTTCAGCTTTGTGGAGGAGTGGACTGAATCCCTGGCT
GCTGTGTTCAACCTCGTCCATGTGGTGTCAGGTGTCTTATTCTACCTGAGCTCAGCTGTC
AACCCCATTATCTATAACCTACTGTCTCGCCGCTTCCAGGCAGCATTCCAGAATGTGATC
TCTTCTTTCCACAAACAGTGGCACTCCCAGCATGACCCACAGTTGCCACCTGCCCAGCGG
AACATCTTCCTGACAGAATGCCACTTTGTGGAGCTGACCGAAGATATAGGTCCCCAATTC
CTATGTCAGTCATCCGTGCACAACTCTCACCTCCCAACAGCCCTCTCTAGTGAACAGATG
TCAAGAACAAACTATCAAAGCTTCCACTTTAACAAAACCTGAATTCTTTCAGAGCTGACT
CTCCTCTATGCCTCAAAACTTCAGAGAGGAACATCCCATAATGTATGCCTTCTCATATGA
AATTAGAGAGGTAGAATGGCTCTTACAACTCATGTACCCATTGCTAGTTTTTTTTTTTTA
ATAAACGTGAAAACTGAGAGTTAGATCTGGTTTCAAAACCCAAGACTGCCTGATTTTTAG
TTATCTTTCCACTATCCTAACTGCCTCATGCCCCTTCACTAGTTCATGCCAAGAACGTGA
CTGGAAAGGCATGGCACCTATACCTTGATTAATTTCCATTAATGGAAATGGTTCGTCCTG
AGTCATCTACGTTCCGAGTCAGGCTGTCACTCCTACTA-3'

Table 3: IGS4A-64-DNA of SEQ ID NO: 9 and SEQ ID NO: 11

5'-
GGCTCAGCTTGAAACAGAGCCTCGTACCAGGGGAGGCTCAGGCCTTGGATTTTAATGTCA
GGGATGGAAAAACTTCAGAATGCTTCCTGGATCTACCAGCAGAAACTAGAAGATCCATTC
CAGAAACACCTGAACAGCACCGAGGAGTATCTGGCCTTCCTCTGCGGACCTCGGCGCAGC
CACTTCTTCCTCCCCGTGTCTGTGGTGTATGTGCCAATTTTTGTGGTGGGGGTCATTGGC
AATGTCCTGGTGTGCCTGGTGATTCTGCAGCACCAGGCTATGAAGACGCCCACCAACTAC
TACCTCTTCAGCCTGGCGGTCTCTGACCTCCTGGTCCTGCTCCTTGGAATGCCCCTGGAG
GTCTATGAGATGTGGCGCAACTACCCTTTCTTGTTCGGGCCCGTGGGCTGCTACTTCAAG
ACGGCCCTCTTTGAGACCGTGTGCTTCGCCTCCATCCTCAGCATCACCACCGTCAGCGTG
GAGCGCTACGTGGCCATCCTACACCCGTTCCGCGCCAAACTGCAGAGCACCCGGCGCCGG
GCCCTCAGGATCCTCGGCATCGTCTGGGGCTTCTCCGTGCTCTTCTCCCTGCCCAACACC
AGCATCCATGGCATCAAGTTCCACTACTTCCCCAATGGGTCCCTGGTCCCAGGTTCGGCC
ACCTGTACGGTCATCAAGCCCATGTGGATCTACAATTTCATCATCCAGGTCACCTCCTTC
CTATTCTACCTCCTCCCCATGACTGTCATCAGTGTCCTCTACTACCTCATGGCACTCAGA
CTAAAGAAAGACAAATCTCTTGAGGCAGATGAAGGGAATGCAAATATTCAAAGACCCTGC
AGAAAATCAGTCAACAAGATGCTGTCTTTGTGGAGGAGTGGAGTGAATCCCTGGCTGCTG
TGTTCAACCTCGTCCATGTGGTGTCAGGTGTCTTCTTCTACCTGAGCTCAGCTGTCAACC
CCATTATCTATAACCTACTGTCTCGCCGCTTCCAGGCAGCATTCCAGAATGTGATCTCTT
CTTTCCACAAACAGTGGCACTCCCAGCATGACCCACAGTTGCCACCTGCCCAGCGGAACA
TCTTCCTGACAGAATGCCACTTTGTGGAGCTGACCGAAGATATAGGTCCCCAATTCCCAT
GTCAGTCATCCATGCACAACTCTCACCTCCCAACAGCCCTCTCTAGTGAACAGATGTCAA
GAACAAACTATCAAAGCTTCCACTTTAACAAAACCTGAATTCTTTCAGAGCTGACTCTCC
TCTATGCCTCAAAACTTCAGAGAGGAACATCCCATAATGTATGCCTTCTCATATGATATT
AGAGAGGTAGAATGGCTCTTACAACTCATGTACCCATTGCTAGTTTTTTTTTTTTAATAA
ACGTGAAAACTGAGAGTTAGATCTGGTTTCAAAACCCAAGACTGCCTGATTTTTAGTTAT
CTTTCCACTATCCTAACTGCCTCATGCCCCTTCACTAGTTCATGCCAAGAACGTGACTGG
AAAGGCATGGCACCTATACCTTGATTAATTTCCATTAATGGAAATGGTTCGTCCTGAGTC
ATCTACGTTCCGAGTCAGGCTGTCACTCCTACTA-3'

Table 4: IGS4A-protein of SEQ ID NO: 2 and SEQ ID NO: 4 (without the three amino acids between brackets).

(MSG)MEKLQNASWIYQQKLEDPFQKHLNSTEEYLAFLCGPRRSHFFLPVSVVYVPIFVVGV
IGNVLVCLVILQHQAMKTPTNYYLFSLAVSDLLVLLLGMPLEVYEMWRNYPFLFGPVGCY
FKTALFETVCFASILSITTVSVERYVAILHPFRAKLQSTRRRALRILGIVWGFSVLFSLP
NTSIHGIKFHYFPNGSLVPGSATCTVIKPMWIYNFIIQVTSFLFYLLPMTVISVLYYLMA
LRLKKDKSLEADEGNANIQRPCRKSVNKMLFVLVLVFAICWAPFHIDRLFFSFVEEWSES
LAAVFNLVHVVSGVFFYLSSAVNPIIYNLLSRRFQAAFQNVISSFHKQWHSQHDPQLPPA
QRNIFLTECHFVELTEDIGPQFPCQSSMHNSHLPTALSSEQMSRTNYQSFHFNKT

Table 5: IGS4B-protein of SEQ ID NO: 6 and SEQ ID NO: 8 (without the three amino acids between brackets).

(MSG)MEKLQNASWIYQQKLEDPFQKHLNSTEEYLAFLCGPRRSHFFLPVSVVYVPIFVVGV
IGNVLVCLVILQHQAMKTPTNYYLFSLAVSDLLVLLLGMPLEVYEMWRNYPFLFGPVGCY
FKTALFETVCFASILSITTVSVERYVAILHPFRAKLQSTRRRALRILGIVWGFSVLFSLP
NTSIHGIKFHYFPNGSLVPGSATCTVIKPMWIYNFIIQVTSFLFYLLPMTVISVLYYLMA
LRLKKDKSLEADEGNANIQRPCRKSVNKMLFVLVLVFAICWAPFHIDRLFFSFVEEWTES
LAAVFNLVHVVSGVLFYLSSAVNPIIYNLLSRRFQAAFQNVISSFHKQWHSQHDPQLPPA
QRNIFLTECHFVELTEDIGPQFLCQSSVHNSHLPTALSSEQMSRTNYQSFHFNKT

Table 6: IGS4A-64-protein of SEQ ID NO: 10 and SEQ ID NO: 12 (without three amino acids between brackets).

(MSG)MEKLQNASWIYQQKLEDPFQKHLNSTEEYLAFLCGPRRSHFFLPVSVVYVPIFVVGV
IGNVLVCLVILQHQAMKTPTNYYLFSLAVSDLLVLLLGMPLEVYEMWRNYPFLFGPVGCY
FKTALFETVCFASILSITTVSVERYVAILHPFRAKLQSTRRRALRILGIVWGFSVLFSLP
NTSIHGIKFHYFPNGSLVPGSATCTVIKPMWIYNFIIQVTSFLFYLLPMTVISVLYYLMA
LRLKKDKSLEADEGNANIQRPCRKSVNKMLSLWRSGVNPWLLCSTSSMWCQVSSST

DESCRIPTION OF THE INVENTION

Definitions

The following definitions are provided to facilitate understanding of certain terms used frequently herein.

"IGS4" refers, among others, to a polypeptide comprising the amino acid sequence set forth in SEQ ID NO: 2 or SEQ ID NO: 4 (IGS4A) and SEQ ID NO: 6 or SEQ ID NO: 8 (IGS4B), or a variant thereof. Particularly preferred are polypeptides of IGS4B.

"Receptor Activity" or "Biological Activity of the Receptor" refers to the metabolic or physiologic function of said IGS4 including similar activities or improved activities or these activities with decreased undesirable side effects. Also included are antigenic and immunogenic activities of said IGS4.

"IGS4-gene" refers to a polynucleotide comprising the nucleotide sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or variants thereof and/or their complements.

"Antibodies" as used herein includes polyclonal and monoclonal antibodies, chimeric, single chain, and humanized antibodies, as well as Fab fragments, including the products of a Fab or other immunoglobulin expression library.

"Isolated" means altered "by the hand of man" from the natural state and/or separated from the natural environment. Thus, if an "isolated" composition or substance that occurs in nature has been "isolated", it has been changed or removed from its original environment, or both. For example, a polynucleotide or a polypeptide naturally present in a living animal is not "isolated", but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated", as the term is employed herein.

"Polynucleotide" generally refers to any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. "Polynucleotides" include, without limitation single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is a mixture of single-and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, "polynucleotide" may also include triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications has been made to DNA and RNA; thus, "polynucleotide"

embraces chemically, enzymatically or metabolically modified forms of polynucleotides as typically found in nature, as well as the chemical forms of DNA and RNA characteristic of viruses and cells. "Polynucleotide" also embraces relatively short polynucleotides, often referred to as oligonucleotides.

"Polypeptide" refers to any peptide or protein comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres. "Polypeptide" refers to short chains, commonly referred to as peptides, oligopeptides or oligomers, and to longer chains, generally referred to as proteins, and/or to combinations thereof. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. "Polypeptides" include amino acid sequences modified either by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well-described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol; cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cystine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York, 1993 and Wold, F., Posttranslational Protein Modifications: Perspectives and Prospects, pgs. 1-12 in POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, 1983; Seifter et al., "Analysis for protein modifications and nonprotein cofactors", Meth. Enzymol. (1990) 182:626-646 and Rattan et al., "Protein Synthesis: Posttranslational Modifications and Aging", Ann. NY Acad. Sci. (1992) 663:48-62.

"Variant" as the term is used herein, is a polynucleotide or polypeptide that differs from a reference polynucleotide or polypeptide respectively, but retains essential properties such as essential biological, structural, regulatory or biochemical propeties. A typical variant of a polynucleotide differs in nucleotide sequence from another, reference polynucleotide. Changes in the nucleotide sequence of the variant may or may not alter the amino acid sequence of a polypeptide encoded by the reference polynucleotide. Nucleotide changes may result in amino acid substitutions, additions, deletions, fusions

and truncations in the polypeptide encoded by the reference sequence, as discussed below. A typical variant of a polypeptide differs in amino acid sequence from another, reference polypeptide. Generally, differences are limited so that the sequences of the reference polypeptide and the variant are closely similar overall and, in many regions, identical. A variant and reference polypeptide may differ in amino acid sequence by one or more substitutions, additions, and deletions in any combination. A substituted or inserted amino acid residue may or may not be one encoded by the genetic code. A variant of a polynucleotide or polypeptide may be a naturally occurring such as an allelic variant, or it may be a variant that is not known to occur naturally. Non-naturally occurring variants of polynucleotides and polypeptides may be made by mutagenesis techniques or by direct synthesis.

"Identity" is a measure of the identity of nucleotide sequences or amino acid sequences. In general, the sequences are aligned so that the highest order match is obtained. "Identity" per se has an art-recognized meaning and can be calculated using published techniques. See, e.g.: (COMPUTATIONAL MOLECULAR BIOLOGY, Lesk, A.M., ed., Oxford University Press, New York, 1988; BIOCOMPUTING: INFORMATICS AND GENOME PROJECTS, Smith, D.W., ed.; Academic Press, New York, 1993; COMPUTER ANALYSIS OF SEQUENCE DATA, PART I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; SEQUENCE ANALYSIS IN MOLECULAR BIOLOGY, von Heinje, G., Academic Press, 1987; and SEQUENCE ANALYSIS PRIMER, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide or polypeptide sequences, the term "identity" is well known to skilled artisans (Carillo, H., and Lipton, D., SIAM J. Applied Math. (1988) 48:1073). Methods commonly employed to determine identity or similarity between two sequences include, but are not limited to, those disclosed in Guide to Huge Computers, Martin J. Bishop, ed., Academic Press, San Diego, 1994, and Carillo, H., and Lipton, D., SIAM J. Applied Math. (1988) 48:1073. Methods to determine identity and similarity are codified in computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., Nucleic Acids Research (1984) 12(1):387), BLASTP, BLASTN, FASTA (Atschul, S.F. et al., J. Molec. Biol. (1990) 215:403). The word "homology" may substitute for the word "identity".

As an illustration, by a polynucleotide having a nucleotide sequence having at least, for example, 95% "identity" to a reference nucleotide sequence of SEQ ID NO: 1 is intended that the nucleotide sequence of the polynucleotide is identical to the reference sequence except that the polynucleotide sequence may include up to five nucleotide differences per each 100 nucleotides of the reference nucleotide sequence of SEQ ID NO: 1. In other words, to obtain a polynucleotide having a nucleotide sequence at least 95% identical to a reference nucleotide sequence, up to any 5% of the nucleotides in the reference sequence may be deleted or substituted with another nucleotide, or a number of nucleotides up to any 5% of the total nucleotides in the reference sequence may be inserted into the reference sequence, or in a number of nucleotides of up to any 5% of the total nucleotides in the

reference sequence there may be a combination of deletion, insertion and substitution. These mutations of the reference sequence may occur at the 5 or 3 terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence.

Similarly, by a polypeptide having an amino acid sequence having at least, for example, 95% "identity" to a reference amino acid sequence of SEQ ID NO: 2 is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the reference amino acid of SEQ ID NO: 2. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to any 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to any 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

Polypeptides of the Invention

In one aspect, the present invention relates to IGS4 polypeptides (or IGS4 proteins). The IGS4 polypeptides include the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 and SEQ ID NO: 8 and the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or deposit no. CBS102222, deposited on September 24, 1999 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands; as well as polypeptides comprising the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands and polypeptides comprising the amino acid sequence which have at least 80% identity to that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and/or the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to said amino acid sequences. Furthermore, those with at least 97%, in particular at least 99%, are highly preferred. Also included within IGS4 polypeptides are polypeptides having the amino acid sequence which has at least 80% identity to the polypeptide having the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 or the polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or deposit

no.CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands over its entire length, and still more preferably at least 90% identity, and even still more preferably at least 95% identity to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8. Furthermore, those with at least 97%, in particular at least 99% are highly preferred. Preferably IGS4 polypeptides exhibit at least one biological activity of the receptor.

In an additional embodiment of the invention, the IGS4 polypeptides may be a part of a larger protein such as a fusion protein. It is often advantageous to include an additional amino acid sequence which contains secretory or leader sequences, pro-sequences, sequences which aid in purification such as multiple histidine residues, sequences which aid in detection such as antigenic peptide tags (such as the haemagglutinin (HA) tag), or an additional sequence for stability during recombinant production.

Fragments of the IGS4 polypeptides are also included in the invention. A fragment is a polypeptide having an amino acid sequence that is the same as part of, but not all of, the amino acid sequence of the aforementioned IGS4 polypeptides. As with IGS4 polypeptides, fragments may be "free-standing," or comprised within a larger polypeptide of which they form a part or region, most preferably as a single continuous region. Representative examples of polypeptide fragments of the invention, include, for example, fragments from about amino acid number 1-20; 21-40, 41-60, 61-80, 81-100; and 101 to the end of IGS4 polypeptide. In this context "about" includes the particularly recited ranges larger or smaller by several, 5, 4, 3, 2 or 1 amino acid at either extreme or at both extremes.

Preferred fragments include, for example, truncation polypeptides having the amino acid sequence of IGS4 polypeptides, except for deletion of a continuous series of residues that includes the amino terminus, or a continuous series of residues that includes the carboxyl terminus or deletion of two continuous series of residues, one including the amino terminus and one including the carboxyl terminus. An example of a truncated polypeptide according to the present invention is the polypetide of SEQ ID NO: 10 and SEQ ID NO: 12, which is encoded by the polynucleotide of SEQ ID NO: 9 respectively SEQ ID NO: 11. Also preferred are fragments characterized by structural or functional attributes such as fragments that comprise alpha-helix and alpha-helix forming regions, beta-sheet and beta-sheet-forming regions, turn and turn-forming regions, coil and coil-forming regions, hydrophilic regions, hydrophobic regions, alpha amphipathic regions, beta amphipathic regions, flexible regions, surface-forming regions, substrate binding region, and high antigenic index regions. Other preferred fragments are biologically active fragments. Biologically active fragments are those that mediate receptor activity, including those with a similar activity or an improved activity, or with a decreased undesirable activity. Also included are those that are antigenic or immunogenic in an animal, especially in a human.

Thus, the polypeptides of the invention include polypeptides having an amino acid sequence at least 80% identical to that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 and/or the

polypeptide having the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands, or fragments thereof with at least 80% identity to the corresponding fragment. Preferably, all of these polypeptide fragments retain the biological activity of the receptor, including antigenic activity. Variants of the defined sequence and fragments also form part of the present invention. Preferred variants are those that vary from the referents by conservative amino acid substitutions -- i.e., those that substitute a residue with another of like characteristics. Typical such substitutions are among Ala, Val, Leu and Ile; among Ser and Thr; among the acidic residues Asp and Glu; among Asn and Gln; and among the basic residues Lys and Arg; or aromatic residues Phe and Tyr. Particularly preferred are variants in which several, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination.

With regard to the polypeptides of the present invention it was also found that they show a high affinity binding for neuromedin U, in particular for neuromedin U-8 (an oligopeptide of 8 amino acids), neuromedin U-23 (an oligopeptide of 23 amino acids) and/or neuromedin U-25 (an oligopeptide of 25 amino acids). In the context of the present invention the term "high affinity" is understood as to describe a ligand binding showing log EC_{50} values of at least below –6.00 (approx. 660 nM), preferably log EC_{50} below –7.00 (approx. 55 nM), more preferably log EC_{50} below –9.00 (approx. 500 pM to 1.2 nM), and most preferably log EC_{50} below –10.00 (approx. 50-100 pM).

Two forms of the neuropeptide neuromedin U, neuromedin U-8 and neuromedin U-25, are described in the literature as uterus stimulating and hypertensive peptides (Minamino et al., 1985, Biochem. Biophys. Res. Commun. 130:1078-1085) being originally isolated from the porcine spinal cord. For neuromedin U-23, an oligopeptide of 23 amino acids, see for example: Okimura et al., Pept. Chem. (1995), Vol. Date 1994, 32:321-324; Salmon et al., J. Biol. Chem. (2000), 275(7), 4549-4554. Neuromedin U (NMU) was subsequently isolated from a number of species, e.g. from rat (NMU-23), human (NMU-25), frog (NMU-25), dog (NMU-8 and NMU-25), rabbit (NMU-25), and chicken (NMU-25). Thus, Domin et al. described the characterization of neuromedin U like immunoreactivity in rat, porcine, guinea pig and human tissue extracts using a specific radioimmunoassay (1986, Biochem. Biophys. Res. Commun. 140:1127-34). The primary structure of neuromedin U 23 from the rat ileum was established by Conlon et al. (1988, J. Neurochem. 51:988-991). Minamino et al. (1988, Biochem. Biophys. Res. Commun. 156:355-360) have isolated rat neuromedin U from the small intestine using mainly immunoaffinity chromatography and radioimmunoassay for pig neuromedin U-8, and the amino acid sequence of rat neuromedin U was determined by microsequence analysis and the structure was confirmed by synthesis. Although the C-terminal heptapeptide amide structure of pig neuromedin U is completely conserved in rat neuromedin U, the remainder of the peptide reveals nine amino acid replacements and two amino acid deletions when compared to pig neuromedin U-25. The distribution, primary structure, and relative biological activity of neuromedin U has been determined also in the frog Rana temporaria by Domin et al. (1989, J. Biol. Chem. 264:20881-20885) showing that the entire

sequence was found to be an icosapentapeptide which displays marked sequence similarity to both porcine and rat neuromedin U. In a further study Domin et al.(1992, Regul. Pept. 41:1-8) have purified an avian homolog of neuromedin U from the chicken. Microsequence analysis characterized the peptide to be 25 amino acid residues long, and chicken neuromedin U showed marked sequence similarity with the porcine peptide at its bioactive C-terminal region. Isolation, structural characterization and pharmacological activity of dog neuromedin U-25 was described by O'Harte et al. (1991 Peptides 12:11-15). Furthermore, for rabbit neuromedin U-25 it was found that it lacks conservation of a posttranslational processing site (Kage et al.,1991 Regul. Pept. 33:191-198); thus, in rabbit neuromedin U, the Arg16-Arg17 dibasic residue processing site that is found in pig and dog neuromedin U-25 is replaced by Arg-Gly, but this potential monobasic processing site is not utilized by cleavage enzyme(s) in the intestine.

Among the species studied the 5 amino acids at the C-terminus of the peptide were found to be almost totally conserved, suggesting that this region is of major importance. Thus, mammalian neuromedins share a common C-terminal sequence "-Phe-Leu-Phe-Arg-Pro-Arg-Asn-amide" which appears to be essential for its biological activities. NMU is distributed both in the gastrointestinal tract and the central nervous System (CNS). In the rat, the highest concentration of neuromedin (NMU) was found in the ileum, followed by the pituitary, hypothalamus, spinal cord, thyroid, and the genitourinary tract. Immunohistochemistry studies showed that NMU immunoreactivity in the gut was only found in nerve fibers, mainly in the myenteric and submucous plexuses, and in the mucosa of all areas except stomach while no NMU immunoreactivity was found in endocrine cells. In the rat brain, NMU immunoreactivity was found in fibers widespread throughout the brain with the exception of the cerebellum. Human and rat genes encoding NMU precursor have been isolated. Both encode NMU at the C-terminus and other potential peptide products in the middle (Lo et al., 1992, J. Mol. Endocrinol. 6:1538-1544; Austin et al., 1995, J. Mol. Endocrinol. 14:157-169). High affinity NMU binding was characterized in rat uterus, and was shown to be sensitive to GTP- -S (Nandha et al., 1993, Endocrinology 133:482-486), suggesting that a receptor for NMU should be a G-protein coupled receptor. Nevertheless, the physiological role of NMU remains largely unknown. Neuromedin U can cause potent contraction of smooth muscle, increase arterial blood pressure, modify intestinal ion transport, and at low doses stimulates the function and growth of the adrenal cortex. NMU was also shown to reduce the blood flow in superior enteric artery and portal vein while increase blood flow slightly in pancreatic tissue.

Furthermore, according to the international patent application WO 90/01330 the neuromedins U-8 and U-25 are described to be suitable in the treatment of disorders of the gastrointestinal tract, e.g. being useful in the selective reduction of blood flow to the gastrointestinal tract, in the treatment of gastrointestinal bleeding and postprandial hypotension.

The IGS4 polypeptides of the present invention have been identified as a G-protein coupled receptor responsive to neuromedin U or ligands sufficiently similar thereto. Thus the IGS4 receptor, in

particular the IGS4B receptor, responsive to neuromedin U will greatly facilitate the understanding of the physiological mechanisms of neuromedin U and other ligands sufficiently similar thereto, as well as of related diseases.

The tissue distribution of the polypeptides of the present invention and the expression levels are shown in the Figures 5-8, from which the skilled artisan can estimate the localisation and relevance of expression. For instance, with regard to the tissue distribution of the polypeptides of the present invention it was found, e.g. by MTE (multiple tissue expression) analysis, Northern blot analysis and Quantitative RT-PCR expression analysis that the IGS4 polypeptides of the present invention particularly are brought to expression with a medium level (relative to expression in testis as 100% in MTE blot, or in spinal cord as 100% in Quantitative RT-PCR analysis, respectively) e.g. in brain, skeletal muscle, cerebellum, thymus, medulla, thyroid, trachea, thalamus, substantia nigra, corpus callosum, caudate nucleus, pons, nucleus accumbens, fetal brain and stomach; and with a relevant level (if being detectable by Quantitative RT-PCR analysis) e.g. in heart, lung, and prostate. For instance, expression levels are considered to be medium if they amount at least 20% of the expression value found for the by far highest expression (set as 100%) in testis or spinal cord. For instance, expression levels are considered to be relevant if expression could be detected at least via Quantitative RT-PCR analysis. It will be appreciated that expression levels indicated for any organ are average values of expression levels in the specific tissues and cell types constituting the organ. Thus, if an expression level is just found to be relevant with respect to an organ, this does not necessarily exclude medium or even high expression levels locally within a specific region, e.g. in a specific tissue and/or cell type, of the organ.

These results suggest that IGS4 polypeptides preferably play a role in the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), in the gastrointestinal system and/or in the cardiovascular system and/or in skeletal muscle and/or in the thyroid, and/or also in lung diseases, immunological diseases and disorders of the genitourinary system.

Thus, in a further embodiment the invention pertains also to an isolated IGS4 polypeptide comprising an amino acid sequence of a neuromedin receptor protein, preferably of a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25. Particularly, the isolated IGS4 polypeptide comprising an amino acid sequence of a neuromedin receptor protein, is a protein exhibiting expression (being at least detectable via Northern and/or MTE and/or Quantitative RT-PCR analysis) in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea. In a variant of this embodiment the invention pertains to an isolated IGS4 polypeptide comprising an amino acid sequence of a neuromedin receptor protein, preferably of a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, said protein exhibiting

expression (being at least detectable via Northern and/or MTE and/or Quantitative RT-PCR analysis) in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea, and said amino acid sequence being selected from the group of amino acid sequence as already defined supra.

The IGS4 polypeptides of the invention can be prepared in any suitable manner. Such polypeptides include isolated naturally occurring polypeptides, recombinantly produced polypeptides, synthetically produced polypeptides, or polypeptides produced by a combination of these methods. Methods for preparing such polypeptides are well known in the art.

Polynucleotides of the Invention

A further aspect of the invention relates to IGS4 polynucleotides. IGS4 polynucleotides include isolated polynucleotides which encode the IGS4 polypeptides (including IGS4A and IGS4B) and fragments, and polynucleotides closely related thereto. More specifically, the IGS4 polynucleotide of the invention includes a polynucleotide comprising the nucleotide sequence contained in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 encoding a IGS4A polypeptide of SEQ ID NO: 2 or of SEQ ID NO: 4 and a IGS4B polypeptide of SEQ ID NO: 6 or of SEQ ID NO: 8 respectively, polynucleotides having the particular sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 and polynucleotides which essentially correspond to the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands.

IGS4 polynucleotides further include polynucleotides comprising a nucleotide sequence that has at least 80% identity over its entire length to a nucleotide sequence encoding the IGS4 polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, a polynucleotide comprising a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 over its entire length and a polynucleotide which essentially correspond to the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands.

In this regard, polynucleotides with at least 90% identity are particularly preferred, and those with at least 95% are especially preferred. Furthermore, those with at least 97% are highly preferred and those with at least 98-99% are most highly preferred, with at least 99% being the most preferred. Also included under IGS4 polynucleotides are a nucleotide sequence which has sufficient identity to a nucleotide sequence contained in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or to the DNA insert contained in the deposit no. CBS102221 or in the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands to hybridize under conditions useable

for amplification or for use as a probe or marker. The invention also provides polynucleotides which are complementary to such IGS4 polynucleotides.

IGS4 of the invention is structurally related to other proteins of the G-protein coupled receptor family, as shown by the results of BLAST searches in the public databases. The amino acid sequence of Table 1 (SEQ ID NO: 2) has about 46 % identity (using BLAST, Altschul S.F. et al. [1997], Nucleic Acids Res. 25:3389-3402) over most of its length (316 amino acid residues) with a human orphan G-protein coupled receptor (Accession # O43664, Tan et al., Genomics 52(2):223-229 (1998). There is 27 % homology (over amino acid residues 61-349) to the rat neurotensin 1 receptor (Accession # P20789 Tanaka K.et al, Neuron 4:847-854 (1990)). The nucleotide sequence of Table 1 (SEQ ID NO: 1) is 63 % identical to an orphan G-protein coupled receptor over nucleotide residues 120-864 (Accession # AF044600, corresponding with the protein sequence O43664). Furthermore, there is 53 % identity to the human growth hormone secretagogue receptor over residues 94-1137 (Howard A.D.et al, Science 273:974-977(1996)). Thus, IGS4 polypeptides and polynucleotides of the present invention are expected to have, inter alia, similar biological functions/properties to their homologous polypeptides and polynucleotides, and their utility is obvious to anyone skilled in the art.

Polynucleotides of the invention can be obtained from natural sources such as genomic DNA. In particular, degenerated PCR primers can be designed that encode conserved regions within a particular GPCR gene subfamily. PCR amplification reactions on genomic DNA or cDNA using the degenerate primers will result in the amplification of several members (both known and novel) of the gene family under consideration (the degenerated primers must be located within the same exon, when a genomic template is used). (Libert et al., Science, 1989, 244: 569-572). Polynucleotides of the invention can also be synthesized using well-known and commercially available techniques (e.g. F.M. Ausubel et al, 2000, Current Protocols in Molecular Biology).

The nucleotide sequence encoding the IGS4 polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 may be identical to the polypeptide encoding sequence contained in SEQ ID NO: 1 (nucleotide number 55 to 1299) or SEQ ID NO: 3 (nucleotide number 64 to 1299), or SEQ ID NO: 5 (nucleotide number 55 to 1299) or SEQ ID NO: 7 (nucleotide number 64 to 1299) respectively, or it may be a different nucleotide sequence, which as a result of the redundancy (degeneracy) of the genetic code might also show alterations compared to the polypeptide encoding sequence contained in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, but also encodes the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8, respectively.

In a further embodiment the invention pertains to an isolated nucleotide sequence encoding an IGS4 neuromedin receptor protein, preferably encoding a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25. Particularly, the isolated nucleotide sequence encodes an IGS4

neuromedin receptor protein which is a protein exhibiting expression (being at least detectable via Northern and/or MTE and/or Quantitative RT-PCR analysis) in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea. In a variant of this embodiment the invention pertains to an isolated nucleotide sequence encoding an IGS4 neuromedin receptor protein, preferably encoding a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, said protein exhibiting expression (being at least detectable via Northern and/or MTE and/or Quantitative RT-PCR analysis) in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea, and said nucleotide sequence being selected from the group of nucleotide sequences as already defined supra.

When the polynucleotides of the invention are used for the recombinant production of the IGS4 polypeptide, the polynucleotide may include the coding sequence for the mature polypeptide or a fragment thereof, by itself; the coding sequence for the mature polypeptide or fragment in reading frame with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, or pro- or prepro- protein sequence, or other fusion peptide portions. For example, a marker sequence which facilitates purification of the fused polypeptide can be encoded. In certain preferred embodiments of this aspect of the invention, the marker sequence is a hexa-histidine peptide, as provided in the pQE vector (Qiagen, Inc.) and described in Gentz et al., Proc. Natl. Acad. Sci USA (1989) 86:821-824, or is an HA tag. The polynucleotide may also contain non-coding 5' and 3' sequences, such as transcribed, non-translated sequences, splicing and polyadenylation signals, ribosome binding sites and sequences that stabilize mRNA.

Further preferred embodiments are polynucleotides encoding IGS4 variants comprising the amino acid sequence of the IGS4 polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 in which several, 5-10, 1-5, 1-3, 1-2 or 1 amino acid residues are substituted, deleted or added, in any combination.

The polynucleotides of the invention can be engineered using methods generally known in the art in order to alter IGS4-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create amino acid substitutions, create new restriction sites, alter modification (e.g. glycosylation or phosphorylation) patterns, change codon preference, produce splice variants, and so forth.

The present invention further relates to polynucleotides that hybridize to the herein above-described sequences. In this regard, the present invention especially relates to polynucleotides which hybridize under stringent conditions to the polynucleotides described above. As herein used, the term "stringent conditions" means hybridization will occur only if there is at least 80%, and preferably at least 90%, and more preferably at least 95%, yet even more preferably at least 97%, in particular at least 99% identity between the sequences.

Polynucleotides of the invention, which are identical or sufficiently identical to a nucleotide sequence contained in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or a fragment thereof, may be used as hybridization probes for cDNA and genomic DNA, to isolate full-length cDNAs and genomic clones encoding IGS4 and to isolate cDNA and genomic clones of other genes (including genes encoding homologs and orthologs from species other than human) that have a high sequence similarity to the IGS4 gene. People skilled in the art are well aware of such hybridization techniques. Typically these nucleotide sequences are 80% identical, preferably 90% identical, more preferably 95% identical to that of the referent. The probes generally will comprise at least 5 nucleotides, and preferably at least 8 nucleotides, and more preferably at least 10 nucleotides, yet even more preferably at least 12 nucleotides, in particular at least 15 nucleotides. Most preferred, such probes will have at least 30 nucleotides and may have at least 50 nucleotides. Particularly preferred probes will range between 30 and 50 nucleotides.

One embodiment, to obtain a polynucleotide encoding the IGS4 polypeptide, including homologs and orthologs from species other than human, comprises the steps of screening an appropriate library under stringent hybridization conditions with a labeled probe having the SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or a fragment thereof, and isolating full-length cDNA and genomic clones containing said polynucleotide sequence. Such hybridization techniques are well known to those of skill in the art. Stringent hybridization conditions are as defined above or alternatively conditions under overnight incubation at 42 °C in a solution comprising: 50% formamide, 5xSSC (150mM NaCl, 15mM trisodium citrate), 50 mM sodium phosphate (pH7.6), 5x Denhardt's solution, 10 % dextran sulfate (w/v), and 20 microgram/ml denatured, sheared salmon sperm DNA, followed by washing the filters in 0.1xSSC at about 65°C.

The polynucleotides and polypeptides of the present invention may be used as research reagents and materials for discovery of treatments and diagnostics to animal and human disease.

Vectors, Host Cells, Expression

The present invention also relates to vectors which comprise a polynucleotide or polynucleotides of the present invention, and host cells which are genetically engineered with vectors of the invention and to the production of polypeptides of the invention by recombinant techniques. Cell-free translation

systems can also be used to produce such proteins using RNAs derived from the DNA constructs of the present invention.

For recombinant production, host cells can be genetically engineered to incorporate expression systems or portions thereof for polynucleotides of the present invention. Introduction of polynucleotides into host cells can be effected by methods described in many standard laboratory manuals, such as Davis et al., BASIC METHODS IN MOLECULAR BIOLOGY (1986) and Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) such as calcium phosphate transfection, DEAE-dextran mediated transfection, transvection, microinjection, cationic lipid-mediated transfection, electroporation, transduction, scrape loading, ballistic introduction or infection.

Representative examples of appropriate hosts include bacterial cells, such as streptococci, staphylococci, E. coli, Streptomyces and Bacillus subtilis cells; fungal cells, such as yeast cells and Aspergillus cells; insect cells such as Drosophila S2 and Spodoptera Sf9 cells; animal cells such as CHO, COS, HeLa, C127, 3T3, BHK, HEK 293 and Bowes melanoma cells; and plant cells.

A great variety of expression systems can be used. Such systems include, among others, chromosomal, episomal and virus-derived systems, e.g., vectors derived from bacterial plasmids, from bacteriophage, from transposons, from yeast episomes, from insertion elements, from yeast chromosomal elements, from viruses such as baculoviruses, papova viruses, such as SV40, vaccinia viruses, adenoviruses, fowl pox viruses, pseudorabies viruses and retroviruses, and vectors derived from combinations thereof, such as those derived from plasmid and bacteriophage genetic elements, such as cosmids and phagemids. The expression systems may contain control regions that regulate as well as engender expression. Generally, any system or vector suitable to maintain, propagate or express polynucleotides to produce a polypeptide in a host may be used. The appropriate nucleotide sequence may be inserted into an expression system by any of a variety of well-known and routine techniques, such as, for example, those set forth in Sambrook et al., MOLECULAR CLONING, A LABORATORY MANUAL (supra).

For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the desired polypeptide. These signals may be endogenous to the polypeptide or they may be heterologous signals, i.e. derived from a different species.

If the IGS4 polypeptide is to be expressed for use in screening assays, generally, it is preferred that the polypeptide be produced at the surface of the cell. In this event, the cells may be harvested prior to use in the screening assay. In case the affinity or functional activity of the IGS4 polypeptide is modified by receptor activity modifying proteins (RAMP), coexpression of the relevant RAMP most likely

at the surface of the cell is preferred and often required. Also in this event harvesting of cells expressing the IGS4 polypeptide and the relevant RAMP prior to use in screening assays is required. If the IGS4 polypeptide is secreted into the medium, the medium can be recovered in order to recover and purify the polypeptide; if produced intracellularly, the cells must first be lysed before the polypeptide is recovered. Membranes expressing the IGS4 polypeptide can be recovered by methods that are well known to a person skilled in the art. In general, such methods include harvesting of the cells expressing the IGS4 polypeptide and homogenization of the cells by a method such as, but not limited to, pottering. The membranes may be recovered by washing the suspension one or several times.

IGS4 polypeptides can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography is employed for purification. Well-known techniques for refolding proteins may be employed to regenerate active conformation when the polypeptide is denatured during isolation and or purification.

Diagnostic Assays

This invention also relates to the use of IGS4 polynucleotides for use as diagnostic reagents. Detection of a mutated form of the IGS4 gene associated with a dysfunction will provide a diagnostic tool that can add to or define a diagnosis of a disease or susceptibility to a disease which results from under-expression, over-expression or altered expression of IGS4. Also in this event co-expression of relevant receptor activity modifying proteins can be required to obtain diagnostic assays of desired quality. Individuals carrying mutations in the IGS4 gene may be detected at the DNA level by a variety of techniques.

Nucleic acids for diagnosis may be obtained from a subject's cells, such as from blood, urine, saliva, tissue biopsy or autopsy material. The genomic DNA may be used directly for detection or may be amplified enzymatically by using PCR or other amplification techniques prior to analysis. RNA or cDNA may also be used in similar fashion. Deletions and insertions can be detected by a change in size of the amplified product in comparison to the normal genotype. Point mutations can be identified by hybridizing amplified DNA to labeled IGS4 nucleotide sequences. Perfectly matched sequences can be distinguished from mismatched duplexes by RNase digestion or by differences in melting temperatures. DNA sequence differences may also be detected by alterations in electrophoretic mobility of DNA fragments in gels, with or without denaturing agents, or by direct DNA sequencing. See, e.g., Myers et al., Science (1985) 230:1242. Sequence changes at specific locations may also be revealed by nuclease protection assays, such as RNase and S1 protection or the chemical cleavage method. See Cotton et al., Proc. Natl. Acad. Sci. USA (1985) 85: 4397-4401. In another embodiment, an array of

oligonucleotide probes comprising the IGS4 nucleotide sequence or fragments thereof can be constructed to conduct efficient screening of e.g., genetic mutations. Array technology methods are well known and have general applicability and can be used to address a variety of questions in molecular genetics including gene expression, genetic linkage, and genetic variability. (See for example: M.Chee et al., Science, Vol 274, pp 610-613 (1996)).

The diagnostic assays offer a process for diagnosing or determining a susceptibility to PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, through detection of mutation in the IGS4 gene by the methods described. According to the present invention, the diagnostic assays offer in particular a process for diagnosing or determining a susceptibility to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

In addition, PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular

diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of the IGS4 polypeptide or IGS4 mRNA. In particular disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also lung diseases, immunological diseases and disorders of the genitourinary system can be diagnosed by methods comprising determining from a sample derived from a subject an abnormally decreased or increased level of the IGS4 polypeptide or IGS4 mRNA. Decreased or increased expression can be measured at the RNA level using any of the methods well known in the art for the quantitation of polynucleotides, such as, for example, PCR, RT-PCR, RNase protection, Northern blotting and other hybridization methods. Assay techniques that can be used to determine levels of a protein, such as an IGS4, in a sample derived from a host are well known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays.

In another aspect, the present invention relates to a diagonostic kit for a disease or suspectibility to a disease, particularly PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal

disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, which comprises:

(a) an IGS4 polynucleotide, preferably the nucleotide sequence of SEQ ID NO: 1. SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7, or a fragment thereof; and/or

(b) a nucleotide sequence complementary to that of (a); and/or

(c) an IGS4 polypeptide, preferably the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or of SEQ ID NO: 8, or a fragment thereof; and/or

(d) an antibody to an IGS4 polypeptide, preferably to the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or of SEQ ID NO: 8; and/or

(e) a RAMP polypeptide required for the relevant biological or antigenic properties of an IGS4 polypeptide

It will be appreciated that in any such kit, (a), (b), (c) (d) or (e) may comprise a substantial component. Preferably the present invention relates to a diagnostic kit for diagnosing or determining a disease or a susceptibility to a disease of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), a disease of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also lung diseases, immunological diseases and disorders of the genitourinary system.

Chromosome Assays

The nucleotide sequences of the present invention are also valuable for chromosome identification. The sequence is specifically targeted to and can hybridize with a particular location on an individual human chromosome. The mapping of relevant sequences to chromosomes according to the present invention is an important first step in correlating those sequences with gene associated disease. Once a sequence has been mapped to a precise chromosomal location, the physical position of the sequence on the chromosome can be correlated with genetic map data. Such data are found, for example, in V. McKusick, Mendelian Inheritance in Man (available on line through Johns Hopkins University Welch Medical Library). The relationship between genes and diseases that have been mapped to the same chromosomal region are then identified through linkage analysis (coinheritance of physically adjacent genes).

The differences in the cDNA or genomic sequence between affected and unaffected individuals can also be determined. If a mutation is observed in some or all of the affected individuals but not in any normal individuals, then the mutation is likely to be the causative agent of the disease.

Antibodies

The polypeptides of the invention or their fragments or analogs thereof, or cells expressing them if required together with relevant RAMP's, may also be used as immunogens to produce antibodies immunospecific for the IGS4 polypeptides. The term "immunospecific" means that the antibodies have substantiall greater affinity for the polypeptides of the invention than their affinity for other related polypeptides in the prior art.

Antibodies generated against the IGS4 polypeptides may be obtained by administering the polypeptides or epitope-bearing fragments, analogs or cells to an animal, preferably a nonhuman, using routine protocols. For preparation of monoclonal antibodies, any technique, which provides antibodies produced by continuous cell line cultures, may be used. Examples include the hybridoma technique (Kohler, G. and Milstein, C., Naure (1975) 256:495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today (1983) 4:72) and the EBV-hybridoma technique (Cole et al., MONOCLONAL ANTIBODIES AND CANCER THERAPY, pp. 77-96, Alan R. Liss, Inc., 1985).

The above-described antibodies may be employed to isolate or to identify clones expressing the polypeptide or to purify the polypeptides by affinity chromatography.

Antibodies against IGS4 polypeptides as such, or against IGS4 polypeptide-RAMP complexes, may also be employed to treat PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock;

sepsis; complication of diabetes mellitus; and gynaecological disorders, among others. Preferably the antibodies of the present invention may be used to treat disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to treat lung diseases, immunological diseases and disorders of the genitourinary system.

Animals

Another aspect of the invention relates to non-human animal-based systems which act as models for disorders arising from aberrant expression or activity of IGS4. Non-human animal-based model systems may also be used to further characterize the activity of the IGS4 gene. Such systems may be utilized as part of screening strategies designed to identify compounds which are capable to treat IGS4 based disorders such as PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders. In particular, the systems may be utilized as part of screening strategies designed to identify compounds which are capable in particular to treat IGS4 based disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to treat lung diseases, immunological diseases and disorders of the genitourinary system. In this way the animal-based models may be used to identify pharmaceutical compounds, therapies and interventions which may be effective in treating disorders of aberrant expression or

activity of IGS4. In addition such animal models may be used to determine the LD_{50} and the ED_{50} in animal subjects. These data may be used to determine the *in vivo* efficacy of potential IGS4 disorder treatments.

Animal-based model systems of IGS4 based disorders, based on aberrant IGS4 expression or activity, may include both non-recombinant animals as well as recombinantly engineered transgenic animals.

Animal models for IGS4 disorders may include, for example, genetic models. Animal models exhibiting IGS4 based disorder-like symptoms may be engineered by utilizing, for example, IGS4 sequences such as those described, above, in conjunction with techniques for producing transgenic animals that are well known to persons skilled in the art. For example, IGS4 sequences may be introduced into, and overexpressed and/or misexpressed in, the genome of the animal of interest, or, if endogenous IGS4 sequences are present, they may either be overexpressed, misexpressed, or, alternatively, may be disrupted in order to underexpress or inactivate IGS4 gene expression.

In order to overexpress or misexpress a IGS4 gene sequence, the coding portion of the IGS4 gene sequence may be ligated to a regulatory sequence which is capable of driving high level gene expression or expression in a cell type in which the gene is not normally expressed in the animal type of interest. Such regulatory regions will be well known to those skilled in the art, and may be utilized in the absence of undue experimentation.

For underexpression of an endogenous IGS4 gene sequence, such a sequence may be isolated and engineered such that when reintroduced into the genome of the animal of interest, the endogenous IGS4 gene alleles will be inactivated, or "knocked-out". Preferably, the engineered IGS4 gene sequence is introduced via gene targeting such that the endogenous IGS4 sequence is disrupted upon integration of the engineered IGS4 gene sequence into the animal's genome. Gene targeting is discussed, below, in this section.

Animals of any species, including, but not limited to, mice, rats, rabbits, squirrels, guinea-pigs, pigs, micro-pigs, goats, and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate animal models of IGS4 related disorders.

Any technique known in the art may be used to introduce a IGS4 transgene into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to pronuclear microinjection (Hoppe, P.C. and Wagner, T.E., 1989, U.S. Pat. No. 4,873,191); retrovirus mediated gene transfer into germ lines (van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152, 1985); gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321, 1989,); electroporation of embryos (Lo, Mol. Cell. Biol. 3:1803-1B14, 1983); and sperm-mediated gene transfer (Lavitrano et al.,

Cell 57:717-723, 1989); etc. For a review of such techniques, see Gordon, Transgenic Animals, Intl. Rev. Cytol.115:171-229, 1989, which is incorporated by reference herein in its entirety.

The present invention provides for transgenic animals that carry the IGS4 transgene in all their cells, as well as animals which carry the transgene in some, but not all their cells, i.e., mosaic animals. (See, for example, techniques described by Jakobovits, Curr. Biol. 4:761-763, 1994) The transgene may be integrated as a single transgene or in concatamers, e.g., head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko, M. et al., Proc. Natl. Acad. Sci. USA 89:6232-6236, 1992).

The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

When it is desired that the IGS4 transgene be integrated into the chromosomal site of the endogenous IGS4 gene, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous IGS4 gene of interest (e.g., nucleotide sequences of the mouse IGS4 gene) are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of, the nucleotide sequence of the endogenous IGS4 gene or gene allele. The transgene may also be selectively introduced into a particular cell type, thus inactivating the endogenous gene of interest in only that cell type, by following, for example, the teaching of Gu et al. (Gu, H. et al., Science 265:103-106, 1994). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant IGS4 gene and protein may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to assay whether integration of the transgene has taken place. The level of mRNA expression of the IGS4 transgene in the tissues of the transgenic animals may also be assessed using techniques which include but are not limited to Northern blot analysis of tissue samples obtained from the animal, in situ hybridization analysis, and RT-PCR. Samples of target gene-expressing tissue, may also be evaluated immunocytochemically using antibodies specific for the target gene transgene product of interest. The IGS4 transgenic animals that express IGS4 gene mRNA or IGS4 transgene peptide (detected immunocytochemically, using antibodies directed against target gene product epitopes) at easily detectable levels may then be further evaluated to identify those animals which display characteristic IGS4 based disorder symptoms.

Once IGS4 transgenic founder animals are produced (i.e., those animals which express IGS4 proteins in cells or tissues of interest, and which, preferably, exhibit symptoms of IGS4 based disorders),

they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound IGS4 transgenics that express the IGS4 transgene of interest at higher levels because of the effects of additive expression of each IGS4 transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the possible need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; breeding animals to different inbred genetic backgrounds so as to examine effects of modifying alleles on expression of the IGS4 transgene and the development of IGS4-like symptoms. One such approach is to cross the IGS4 transgenic founder animals with a wild type strain to produce an F1 generation that exhibits IGS4 related disorder-like symptoms, such as those described above. The F1 generation may then be inbred in order to develop a homozygous line, if it is found that homozygous target gene transgenic animals are viable.

Vaccines

Another aspect of the invention relates to a method for inducing an immunological response in a mammal which comprises administering to (for example by inoculation) the mammal the IGS4 polypeptide, or a fragment thereof, if required together with a RAMP polypeptide, adequate to produce antibody and/or T cell immune response to protect said animal from PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders, among others. Yet another aspect of the invention relates to a method of inducing

immunological response in a mammal which comprises delivering the IGS4 polypeptide via a vector directing expression of the IGS4 polynucleotide in vivo in order to induce such an immunological response to produce antibody to protect said animal from diseases. In particular the invention relates to a method for inducing an immunological response in a mammal which comprises inoculating the mammal with the IGS4 polypeptide, or a fragment thereof, if required together with a RAMP polypeptide, adequate to produce antibody and/or T cell immune response to protect said animal from disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also from lung diseases, immunological diseases and disorders of the genitourinary system.

A further aspect of the invention relates to an immunological/vaccine formulation (composition) which, when introduced into a mammalian host, induces an immunological response in that mammal to an IGS4 polypeptide wherein the composition comprises an IGS4 polypeptide or IGS4 gene. Such immunological/vaccine formulations (compositions) may be either therapeutic immunological/vaccine formulations or prophylactic immunological/vaccine formulations. The vaccine formulation may further comprise a suitable carrier. Since the IGS4 polypeptide may be broken down in the stomach, it is preferably administered parenterally (including subcutaneous, intramuscular, intravenous, intradermal etc. injection). Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents or thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example, sealed ampoules and vials and may be stored in a freeze-dried condition requiring only the addition of the sterile liquid carrier immediately prior to use. The vaccine formulation may also include adjuvant systems for enhancing the immunogenicity of the formulation, such as oil-in water systems and other systems known in the art. The dosage will depend on the specific activity of the vaccine and can be readily determined by routine experimentation.

Screening Assays

The IGS4 polypeptide of the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the receptor polypeptide of the present invention. Thus, polypeptides of the invention may also be used to assess the binding of small molecule substrates and ligands in, for example, cells, cell-free preparations, chemical libraries, and natural product mixtures. These substrates and ligands may be natural substrates and ligands or may be structural or functional mimetics.

IGS4 polypeptides are responsible for biological functions, including pathologies. Accordingly, it is desirable to find compounds and drugs which stimulate IGS4 on the one hand and which can inhibit the

function of IGS4 on the other hand. In general, agonists are employed for therapeutic and prophylactic purposes for such conditions as PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and gynaecological disorders. Antagonists may be employed for a variety of therapeutic and prophylactic purposes for such conditions as PNS, psychiatric and CNS disorders, including schizophrenia, episodic paroxysmal anxiety (EPA) disorders such as obsessive compulsive disorder (OCD), post traumatic stress disorder (PTSD), phobia and panic, major depressive disorder, bipolar disorder, Parkinson's disease, general anxiety disorder, autism, delirium, multiple sclerosis, Alzheimer disease/dementia and other neurodegenerative diseases, severe mental retardation, dyskinesias, Huntington's disease, Tourett's syndrome, tics, tremor, dystonia, spasms, anorexia, bulimia, stroke, addiction/dependency/craving, sleep disorder, epilepsy, migraine; attention deficit/hyperactivity disorder (ADHD); cardiovascular diseases, including heart failure, angina pectoris, arrhythmias, myocardial infarction, cardiac hypertrophy, hypotension, hypertension – e.g. essential hypertension, renal hypertension, or pulmonary hypertension, thrombosis, arteriosclerosis, cerebral vasospasm, subarachnoid hemorrhage, cerebral ischemia, cerebral infarction, peripheral vascular disease, Raynaud's disease, kidney disease – e.g. renal failure; dyslipidemias; obesity; emesis; gastrointestinal disorders, including irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastroesophagal reflux disease (GERD), motility disorders and conditions of delayed gastric emptying, such as post operative or diabetic gastroparesis, and diabetes, ulcers – e.g. gastric ulcer; diarrhoea; other diseases including osteoporosis; inflammations; infections such as bacterial, fungal, protozoan and viral infections, particularly infections caused by HIV-1 or HIV-2; pain; cancers; chemotherapy induced injury; tumor invasion; immune disorders; urinary retention; asthma; allergies; arthritis; benign prostatic hypertrophy; endotoxin shock; sepsis; complication of diabetes mellitus; and

gynaecological disorders. Particularly, the present invention may be employed in a screening process for compounds which bind the receptor and which activate (agonists) or inhibit activation of (antagonists) the IGS4 neuromedin receptor protein, preferably the mammalian IGS4 neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25. These screening assays are particularly suitable for screening compounds which are effective with regard to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

In general, such screening procedures involve producing appropriate cells, which express the receptor polypeptide of the present invention on the surface thereof and, if essential co-expression of RAMP's at the surface thereof. Such cells include cells from mammals, yeast, Drosophila or E. coli. Cells expressing the receptor (or cell membrane containing the expressed receptor) are then contacted with a test compound to observe binding, or stimulation or inhibition of a functional response.

One screening technique includes the use of cells which express the receptor of this invention (for example, transfected CHO cells) in a system which measures extracellular pH, intracellular pH, or intracellular calcium changes caused by receptor activation. In this technique, compounds may be contacted with cells expressing the receptor polypeptide of the present invention. A second messenger response, e.g., signal transduction, pH changes, or changes in calcium level, is then measured to determine whether the potential compound activates or inhibits the receptor.

Another method involves screening for receptor inhibitors by determining modulation of a receptor-mediated signal, such as cAMP accumulation and/or adenylate cyclase activity. Such a method involves transfecting an eukaryotic cell with the receptor of this invention to express the receptor on the cell surface. The cell is then exposed to an agonist to the receptor of this invention in the presence of a potential antagonist. If the potential antagonist binds the receptor, and thus inhibits receptor binding, the agonist-mediated signal will be modulated.

Another method for detecting agonists or antagonists for the receptor of the present invention is the yeast-based technology as described in U.S. Patent 5,482,835, incorporated by reference herein.

The assays may simply test binding of a candidate compound wherein adherence to the cells bearing the receptor is detected by means of a label directly or indirectly associated with the candidate compound or in an assay involving competition with a labeled competitor. Further, these assays may test whether the candidate compound results in a signal generated by activation of the receptor, using detection systems appropriate to the cells bearing the receptor at their surfaces. Inhibitors of activation

are generally assayed in the presence of a known agonist and the effect on activation by the agonist by the presence of the candidate compound is observed.

Thus candidate compounds may be screened which show ligand binding to the IGS4 receptors of the present invention. In the context of the present invention the term "ligand binding" is understood as to describe compounds with affinity to the IGS4 receptors showing log EC_{50} values of at least below –6.00 (approx. 660 nM), preferably log EC_{50} below –7.00 (approx. 55 nM), more preferably log EC_{50} below –9.00 (approx. 500 pM to 1.2 nM), and most preferably log EC_{50} below –10.00 (approx. 50-100 pM).

Thus in one aspect the invention concerns a method of determining whether a substance is a potential ligand of IGS4 receptor comprising

(a) contacting cells expressing one of the IGS4 neuromedin receptors defined supra or one of the receptors of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, or contacting a receptor membrane preparation comprising one of said IGS4 neuromedin receptors defined supra or one of the receptors of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 with labeled neuromedin U in the presence and in the absence of the substance; and

(b) measuring the binding of neuromedin U to IGS4.

Further, the assays may simply comprise the steps of mixing a candidate compound with a solution containing an IGS4 polypeptide to form a mixture, measuring the IGS4 activity in the mixture, and comparing the IGS4 activity of the mixture to a standard.

The IGS4 cDNA, protein and antibodies to the protein may also be used to configure assays for detecting the effect of added compounds on the production of IGS4 mRNA and protein in cells. For example, an ELISA may be constructed for measuring secreted or cell associated levels of IGS4 protein using monoclonal and polyclonal antibodies by standard methods known in the art, and this can be used to discover agents which may inhibit or enhance the production of IGS4 (also called antagonist or agonist, respectively) from suitably manipulated cells or tissues. Standard methods for conducting screening assays are well known in the art.

Examples of potential IGS4 antagonists include antibodies or, in some cases, oligonucleotides or proteins which are closely related to the ligand of the IGS4, e.g., a fragment of the ligand, or small molecules which bind to the receptor but do not elicit a response, so that the activity of the receptor is prevented.

Thus in another aspect, the present invention relates to a screening kit for identifying agonists, antagonists, ligands, receptors, substrates, enzymes, etc. for IGS4 polypeptides; or compounds which decrease, increase and/or otherwise enhance the production of IGS4 polypeptides, which comprises:

(a) an IGS4 polypeptide, preferably that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8;

(b) a recombinant cell expressing an IGS4 polypeptide, preferably that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8;

(c) a cell membrane expressing an IGS4 polypeptide; preferably that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8; or

(d) antibody to an IGS4 polypeptide, preferably that of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.

It will be appreciated that in any such kit, (a), (b), (c) or (d) may comprise a substantial component.

Prophylactic and Therapeutic Methods

This invention provides methods of treating abnormal conditions related to both an excess of and insufficient amounts of IGS4 activity.

If the activity of IGS4 is in excess, several approaches are available. One approach comprises administering to a subject an inhibitor compound (antagonist) as hereinabove described along with a pharmaceutically acceptable carrier in an amount effective to inhibit activation by blocking binding of ligands to the IGS4, or by inhibiting interaction with a RAMP polypeptide or a second signal, and thereby alleviating the abnormal condition.

In another approach, soluble forms of IGS4 polypeptides still capable of binding the ligand in competition with endogenous IGS4 may be administered. Typical embodiments of such competitors comprise fragments of the IGS4 polypeptide.

In still another approach, expression of the gene encoding endogenous IGS4 can be inhibited using expression-blocking techniques. Known such techniques involve the use of antisense sequences, either internally generated or separately administered. See, for example, O'Connor, J Neurochem (1991) 56:560 in Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression, CRC Press, Boca Raton, Florida USA (1988). Alternatively, oligonucleotides, which form triple helices with the gene, can be supplied. See, for example, Lee et al., Nucleic Acids Res (1979) 6:3073; Cooney et al., Science (1988) 241:456; Dervan et al, Science (1991) 251:1360. These oligomers can be administered per se or the relevant oligomers can be expressed in vivo. Synthetic antisense or triplex oligonucleotides may comprise modified bases or modified backbones. Examples of the latter include methylphosphonate, phosphorothioate or peptide nucleic acid backbones. Such backbones are incorporated in the antisense or triplex oligonucleotide in order to provide protection from degradation by nucleases and are well known in the art. Antisense and triplex molecules synthesized with these or other modified backbones also form part of the present invention.

In addition, expression of the IGS1 polypeptide may be prevented by using ribozymes specific to the IGS1 mRNA sequence. Ribozymes are catalytically active RNAs that can be natural or synthetic (see for example Usman, N, et al., Curr. Opin. Struct. Biol (1996) 6(4), 527-33.) Synthetic ribozymes can be designed to specifically cleave IGS1 mRNAs at selected positions thereby preventing translation of the IGS1 mRNAs into functional polypeptide. Ribozymes may be synthesized with a natural ribose phosphate backbone and natural bases, as normally found in RNA molecules. Alternatively the ribosymes may be synthesized with non-natural backbones to provide protection from ribonuclease degradation, for example, 2'-O-methyl RNA, and may contain modified bases.

For treating abnormal conditions related to an under-expression of IGS4 and its activity, several approaches are also available. One approach comprises administering to a subject a therapeutically effective amount of a compound which activates IGS4, i.e., an agonist as described above, in combination with a pharmaceutically acceptable carrier, to thereby alleviate the abnormal condition. Alternatively, gene therapy may be employed to effect the endogenous production of IGS4 by the relevant cells in the subject. For example, a polynucleotide of the invention may be engineered for expression in a replication defective retroviral vector, as discussed above. The retroviral expression construct may then be isolated and introduced into a packaging cell transduced with a retroviral plasmid vector containing RNA encoding a polypeptide of the present invention such that the packaging cell now produces infectious viral particles containing the gene of interest. These producer cells may be administered to a subject for engineering cells in vivo and expression of the polypeptide in vivo. For overview of gene therapy, see Chapter 20, Gene Therapy and other Molecular Genetic-based Therapeutic Approaches, (and references cited therein) in Human Molecular Genetics, Strachan T. and Read A.P., BIOS Scientific Publishers Ltd (1996).

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

Formulation and Administration

Peptides, such as the soluble form of IGS4 polypeptides, and agonists and antagonist peptides or small molecules, may be formulated in combination with a suitable pharmaceutical carrier. Such formulations comprise a therapeutically effective amount of the polypeptide or compound, and a pharmaceutically acceptable carrier or excipient. Formulation should suit the mode of administration, and is well within the skill of the art. The invention further relates to pharmaceutical packs and kits comprising one or more containers filled with one or more of the ingredients of the aforementioned compositions of the invention.

Polypeptides and other compounds of the present invention may be employed alone or in conjunction with other compounds, such as therapeutic compounds.

Preferred forms of systemic administration of the pharmaceutical compositions include injection, typically by intravenous injection. Other injection routes, such as subcutaneous, intramuscular, or intraperitoneal, can be used. Alternative means for systemic administration include transmucosal and transdermal administration using penetrants such as bile salts or fusidic acids or other detergents. In addition, if properly formulated in enteric or encapsulated formulations, oral administration may also be possible.

The dosage range required depends on the choice of peptide or compound, the route of administration, the nature of the formulation, the nature of the subject's condition, and the judgment of the attending practitioner. Suitable dosages are in the range of 0.1-100 μg/kg of subject. Wide variations in the needed dosage, however, are to be expected in view of the variety of compounds available and the differing efficiencies of various routes of administration. For example, oral administration would be expected to require higher dosages than administration by intravenous injection. Variations in these dosage levels can be adjusted using standard empirical routines for optimization, as is well understood in the art.

Polypeptides used in treatment can also be generated endogenously in the subject, in treatment modalities often referred to as "gene therapy" as described above. Thus, for example, cells from a subject may be engineered with a polynucleotide, such as a DNA or RNA, to encode a polypeptide ex vivo, and for example, by the use of a retroviral plasmid vector. The cells are then introduced into the subject.

The following examples are only intended to further illustrate the invention in more detail, and therefore these examples are not deemed to restrict the scope of the invention in any way.

EXAMPLE 1. THE CLONING OF CDNA ENCODING A NOVEL G PROTEIN-COUPLED RECEPTOR.

Example 1a. Homology PCR cloning of a genomic fragment encoding a novel G-protein coupled receptor (GPCR).

A PCR based homology cloning strategy was used to isolate partial genomic DNA sequences encoding novel G-protein coupled receptors. Forward (F22) and reverse (R44 and R46) degenerate PCR primers were designed in conserved areas of the neurotensin receptor gene family (Vita N. et al. [1993] Febs Lett. 317, 139-142; Vita N. et al. [1998] Eur. J. Pharmacol. 360, 265-272) within transmembrane domains 1 (TM1) and 3 (TM3) and at the boundary between TM3 and intracellular loop 2 (I2):

F22 (TM1):
5'-CTCATCTTCGCGGTGGGC(A or G)C(A,C,G or T)G(C or T)(A,C,G or T)GG-3' (SEQ ID NO: 13)

R44 (TM3/I2):
5'-GGCCAGGCAGCGCTCCGCGCT(C or Inosine)A(A or G)(A,C,G or T)C(C or T)(A,C,G or T)GC(A,G or T)-3' (SEQ ID NO: 14)

R46 (TM3):
5'-GAA(A or G)TA(A or G)TAGCC(A or G)CG(A or G)CAGCC(A or T)-3' (SEQ ID NO: 15)

In order to suppress amplification of known members of the neurotensin receptor family, the 3' ultimate nucleotide position of primer R44 was chosen in such a way that is was not complementary to the corresponding position of both NTR1 and NTR2 cDNA. The primary PCR reaction was carried out in a 60µl volume and contained 100ng human genomic DNA (Clontech), 6 µl GeneAmp™ 10 x PCR buffer II (100mM Tris-HCl pH 8.3; 500 mM KCl, Perkin Elmer), 3.6 µl 25 mM $MgCl_2$, 0.36 µl dNTPs (25mM of each dNTP), 1.5 units AmpliTaq-Gold™ polymerase (Perkin Elmer) and 30 pmoles of each of the degenerated forward (F22) and reverse primer (R44). Reaction tubes were heated at 95°C for 10 min and then subjected to 35 cycles of denaturation (95°C, 1 min), annealing (55°C, 2 min) and extension (72°C, 3min). Finally reaction tubes were heated for 10 min at 72°C.

For the semi-nested PCR reaction 1 µl of a 1/50 dilution of the primary PCR reaction was used as a template using the degenerate forward and reverse primers F22 and R46 respectively. The semi-nested PCR reaction was carried out under the same conditions as the primary PCR reaction.

Semi-nested PCR reaction products were size fractionated on an agarose gel and stained with ethidium bromide. Although a fragment of ± 220 bp was expected, only a fragment of ± 120 bp was visible. This fragment was purified from gel using the Qiaex-II™ purification kit (Qiagen) and ligated into the pGEM-T plasmid according to the procedure recommended by the supplier (pGEM-T kit, Promega). The recombinant plasmids thus produced were used to transform competent E. coli SURE™ 2 bacteria (Stratagene). Transformed cells were plated on LB agar plates containing ampicillin (100 µg/ml), IPTG (0.5 mM) and X-gal (50 µg/ml). Plasmid DNA was purified from mini-cultures of individual colonies using

a Qiagen-tip 20 miniprep kit (Qiagen). DNA sequencing reactions were carried out on the purified plasmid DNA with the ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit (PE-ABI), using insert-flanking primers.

Table 7: Overview of oligo primers used.

SEQ ID NO: 13	F22: 5'-CTCATCTTCGCGGTGGGC(A or G)C(A,C,G or T)G(C or T)(A,C,G or T)GG-3'
SEQ ID NO: 14	R44: 5'-GGCCAGGCAGCGCTCCGCGCT(C or Inosine)A(A or G)(A,C,G or T)C(C or T)(A,C,G or T)GC(A,G or T)-3'
SEQ ID NO: 15	R46: 5'-GAA(A or G)TA(A or G)TAGCC(A or G)CG(A or G)CAGCC(A or T)-3'
SEQ ID NO: 16	AP1: 5'-CCATCCTAATACGACTCACTATAGGGC-3'
SEQ ID NO: 17	AP2: 5'-ACTCACTATAGGGCTCGAGCGGC-3'
SEQ ID NO: 18	IGS4R1: 5'GGATCCCAAATAAGAAAGGGTAGTTGC-3'
SEQ ID NO: 19	IGS4R2: 5'AAAGGGTAGTTGCGCCACATCTCATAGAC-3'
SEQ ID NO: 20	IGS4F5: 5'AGGTCTATGAGATGTGGCGCAACTACCCT-3'
SEQ ID NO: 21	IGS4F6: 5'ATGTGGCGCAACTACCCTTTCTTATTTGGG-3'
SEQ ID NO: 22	R74: 5'-CGGAAGTTGGCGGACACG(A or G)(A,C or G)(A or G)TT(A or G)TA-3'
SEQ ID NO: 23	IGS4F7: 5'-GCTCAGCTTGAAACAGAGCCTCGTACC-3'
SEQ ID NO: 24	IGS4F8: 5'-CCATGTGGATCTACAATTTCATCATCC-3'
SEQ ID NO: 25	IGS4F9: 5'-AAGACAAATCTCTTGAGGCAGATGAAGGG-3'
SEQ ID NO: 26	IGS4F10: 5'-GATGCTGTTTGTCTTGGTCTTAGTGTTTGC-3'
SEQ ID NO: 27	IGS4R5: 5'-GGATGATGAAATTGTAGATCCACATGGGC-3'
SEQ ID NO: 28	IGS4R6: 5'-TGTGGAGAAGTCTCTCAAAGTGTGG-3'
SEQ ID NO: 29	IGS4R7: 5'-TAGTAGGAGTGACAGCCTGACTCGGAACG-3'
SEQ ID NO: 30	IGS4R8: 5'-AACGTAGATGACTCAGGACGAACCATTTCC-3'
SEQ ID NO: 31	IGS4F11: 5'-TCGTACCAGGGGAGGCTCAGGC-3'

Sequencing reaction products were purified via EtOH/NaOAc precipitation and analyzed on an ABI 377 automated sequencer.

Sequence analysis of the insert of clone HNT1552 showed that it potentially encoded part of a novel member of the GPCR family. We refer to this novel GPCR sequence as IGS4.

Example 1b. Cloning of cDNA fragments containing the complete IGS4 coding sequence.

The complete coding sequence of IGS4 cDNA was obtained via both RACE analysis (rapid amplification of cDNA ends) and RT-PCR amplification. 5'- and 3' RACE PCR reactions were performed on Marathon-Ready™ cDNA from human brain or testis (Clontech n° 7400-1 and 7414-1 respectively), using the adaptor primers 1 and 2 (AP1: SEQ ID NO: 16 ; AP2: SEQ ID NO: 17) provided with the

Marathon™ cDNA amplification kit (Clontech K1802-1) and IGS4 specific primers. PCR RACE reactions were performed according to the instructions of the Marathon-Ready™ cDNA user manual provided by Clontech. RACE products were separated on agarose gel, visualized with ethidium bromide and blotted onto Hybond N$^+$ membranes. Blots were prehybridized at 65°C for 2 h in modified Church buffer (0.5M phosphate, 7% SDS, 10 mM EDTA) and then hybridised overnight at 65°C in the same buffer containing 2 x 10^6 cpm / ml of a ^{32}P-labelled IGS4 cDNA probe. IGS4 cDNA probes were radiolabelled via random primed incorporation of [α-^{32}P]dCTP to a specific activity of > 10^9 cpm/µg using the Prime-It II kit™ (Stratagene) according to the instructions provided by the supplier. Hybridized blots were washed at high stringency (2 x 30 min at room temperature in 2 x SSC/ 0.1% SDS, followed by 2 washes of 40 min at 65°C in 0.1 x SSC, 0.1% SDS) and autoradiographed overnight. Hybridizing fragments were purified from a preparative gel, cloned into the pGEM-T vector and sequenced as described above.

An initial round of semi-nested 5' RACE analysis on human brain cDNA using the IGS4 specific primers IGS4R1 (SEQ ID NO: 18) and IGS4R2 (SEQ ID NO: 19)(designed on the DNA sequence of clone HNT1552) yielded clones HNT1886 and HNT1887 (Fig.1). These clones extended the IGS4 cDNA sequence upstream up to and beyond the putative start of translation codon. Likewise an initial round of 3' RACE analysis on human brain cDNA using IGS4 specific primers IGS4F5 (SEQ ID NO: 20) and IGS4F6 (SEQ ID NO: 21) yielded clones HNT1874-1878 and HNT1902-1903 (Fig.1). These clones extended the known IGS4 cDNA at the 3' end.

All sequences obtained at this point were assembled into a single contig which contained a long open reading frame, encoding part of a novel protein that was most similar to human orphan receptor FM-3 (Tan et al., Genomics 52, 223-229 [1998], GenBank accession n° AF044600 and AF044601). To investigate the RNA expression profile of IGS4, a Master Blot™ membrane (Clontech cat n° 7770-1) containing RNA from different human tissues was hybridized to the ^{32}P-labelled insert of clone HNT1903 under the conditions recommended by the supplier. The strongest hybridization was obtained with testis RNA whereas much weaker signals were obtained in prostate, stomach, spinal cord, hippocampus, medulla oblongata, thyroid gland, thymus, lung and trachea.

Since the contig sequence did not yet contain the complete IGS4 coding sequence we set up an RT-PCR homology cloning experiment on human total brain RNA using IGS4 specific primer IGS4F6 (SEQ ID NO: 21) and a degenerated primer (R74, SEQ ID NO: 22), which was designed in a conserved area (at the TM7/C-terminal intracellular part) of the GPCR subfamily composed of the neurotensin receptors 1 and 2, the growth hormone secretagogue receptor (Howard A.D. et al.[1996] Science 273, 974-977) and the orphan GPCR FM-3 and GPR38 (McKee K.K. et al.[1997] Genomics 46, 426-434). RT-PCR reactions were carried out in a 50 µl volume on 500 ng total RNA from human brain using the Titan™ One Tube RT-PCR System (Boehringer catalogue n° 1,888,382) according to the recommendations of the supplier. Briefly, RT-PCR conditions were as follows: reverse transcription for 45 min at 55°C; 2 min denaturation at 94°C, followed by a touch-down PCR reaction of 20 cycles (30 sec denaturation at 94°C, 30 sec annealing at 60°C [-0.25°C/cycle] and 2 min extension at 68°C) and an additional round of 30 PCR cycles (30 sec denaturation at 94°C, 30 sec annealing at 55°C and 3 min [+ 5 sec/cycle] extension at 68°C). This was concluded with an extra extension step of 7 min at 68°C.

Reaction products were analyzed via Southern blotting using the radiolabelled insert of clone HNT1903. A fragment of ± 690 bp that hybridized to the probe was purified from the gel (QiaexII™, Qiagen) and cloned into the pGEM-T vector yielding clones HNT2210-2212. Sequence analysis of these clones allowed to extend the existing IGS4 cDNA contig in the 3' direction.

Since the extended IGS4 cDNA contig still did not yet contain a translational stop codon, additional IGS4 specific 3' RACE primers were designed (IGS4F7-10, SEQ ID NO: 23-26)). Nested or semi-nested 3' RACE reactions were carried out on Marathon Ready™ cDNA from human testis (Clontech 7414-1). IGS4 specific bands (as assessed via Southern blot analysis using an IGS4 specific probe) were cloned into pGEM-T. This yielded clones HNT2289-90 (AP1/IGS4F5->AP2/IGS4F9), HNT2293-2295 (AP1/IGS4F6->AP2/IGS4F9), HNT2296-2297 (AP1/IGS4F7->AP2/IGS4F9), HNT2308-2310 (AP1/IGS4F8->AP2/IGS4F10) HNT2253 (AP1/IGS4F7->AP1/IGS4F5). An additional 5' RACE PCR reaction carried out on testis Marathon Ready™ cDNA yielded clones HNT2279-2281 (AP2/IGS4R6->AP2/IGS4R5). (note: AP1/IGS4F5->AP2/IGS4F9 e.g. indicates that clones were generated from an IGS4 specific fragment obtained after the primary RACE PCR reaction [using primer pair AP1/IGS4F5] was nested with primer pair AP2/IGS4F9).

Sequence analysis of these clones allowed to extend the existing IGS4 cDNA contig further in the 3' direction although the end of the IGS4 coding sequence was not yet been reached. A computer-assisted homology search (Blastn; Altschul S.F. et al., Nucleic Acids Res. (1997) 25:3389-3402) of the IGS4 contig DNA sequence against the expressed sequence tag (EST) database (dbest) showed the presence of an EST sequence (accession n° N45474) which overlapped with the 3' end of the IGS4 contig (near 100 % identity in the overlap area). EST N45474 further extended the IGS4 DNA contig at the 3' end into a translational stop codon and into the 3' untranslated region (3'-UTR). In addition another set of ESTs was identified which all covered the 3'-UTR of the IGS4 mRNA (Fig.2). Additional IGS4 specific primers (IGS4R7-8, SEQ ID NO: 29-30)) were designed within the 3'-UTR of these ESTs. Primary PCR reactions were carried out on Marathon Ready™ cDNA from human testis using various combinations of the IGS4F7 (SEQ ID NO: 23), IGS4F11 (SEQ ID NO: 31) and IGS4R7-8 (SEQ ID NO: 29-30) primers. PCR tubes were heated for 2 min at 95°C and then subjected to 35 cycles of denaturation (95°C, 30 sec), annealing (65°C, 30 sec) and extension (72°C, 1 min 30 sec). Finally the reactions tubes were heated at 72°C for 10 min. Nested PCR reactions were also carried out under the same conditions. DNA fragments of ± 1630 bp were purified from gel and cloned into the pGEM-T vector. The following clones were obtained: HNT2311, HNT2312 and HNT2317 (IGS4F7/IGS4R7->IGS4F11/IGS4R8); HNT2313, HNT2324, HNT2326 and HNT2328 (IGS4F11->IGS4R8); HNT2314, HNT2315 and HNT2322 (IGS4F11->R7). Clone HNT2363 was obtained from a purified 1630 bp PCR fragment, that was amplified from human testis Marathon Ready™ cDNA using the IGS4F11/R7 primer pair under the following slightly modified conditions. After an initial denaturation of 2 min at 94°C, PCR tubes were subjected to 15 cycles of denaturation [15 sec, 94°C], annealing [30 sec, 65°C] and extension [2 min, 72°C] followed by another 20 cycles of denaturation [15 sec, 94°C], annealing [30 sec, 65°C] and extension [2 min, 72°C; +10sec/cycle]. There was a final extension step of 7 min at 72°C. Sequence analysis of these clones allowed to assemble an IGS4 cDNA consensus sequence (Fig.1).

Close inspection of all clones showed that they actually were of 2 sequence types, which differed at 5 nucleotide positions. These variant sequences correspond to a polymorphism within the human population. We refer to these different cDNA types as IGS4ADNA (SEQ ID NO: 1 and SEQ ID NO: 3) and IGS4BDNA (SEQ ID NO: 5 and SEQ ID NO: 7). The consensus sequence contained a long open reading frame that contained two in-frame start codons (positions 55-57 (SEQ ID NO: 1 and SEQ ID NO: 5) and 64-66 (SEQ ID NO: 3 and SEQ ID NO: 7) in IGS4ADNA and IGS4BDNA), predicting a protein of either 415 (SEQ ID NO: 2 and SEQ ID NO: 6) or 412 (SEQ ID NO: 4 and SEQ ID NO: 8) amino acids, which showed good homology to GPCR proteins. Hydropathy analysis (Kyte J. et al.[1982] J. Mol. Biol. 157: 105-132; Klein P.et al.[1985] Biochim. Biophys. Acta 815:468-476) of the protein also indicated the presence of 7 transmembrane domains. Since the first ATG initiator codon is within a weak "Kozak" translation initiation context and the second one is in a strong Kozak context, it is likely that the IGS4A/B protein starts at the second methionine and is 412 amino acids long (Kozak M. [1999] Gene 234, 187-208). However some (or even exlusive) initiation at the first ATG cannot be excluded. Among the five polymorphic nucleotides, four (positions 947, 999, 1202 and 1216 in IGS4A/BDNA) resulted in a switch in the encoded amino acid residue, whereas the fifth (pos 1381 in IGS4A/BDNA) was within the 3'-UTR. The respective predicted protein sequences are referred to as IGS4APROT (SEQ ID NO: 2 and SEQ ID NO: 4) and IGS4BPROT (SEQ ID NO: 6 and SEQ ID NO: 8). (note 1: the sequence of IGS4APROT and IGS4BPROT in this document is represented as the longest possible (415 amino acids) sequence but it is understood that the actual protein might be 3 amino acids shorter at the amino-terminus; for this reason the first 3 amino acids of IGS4APROT and IGS4BPROT in Table 4 and 5 have been bracketed) (note 2: In this document IGS4 refers to the IGS4 sequence in general, irrespective of the particular allelic type). Homology searches of the IGS4 protein sequence against public domain protein databanks showed best homology to the human orphan GPCR FM-3 (accession no O43664, Tan C.P., et al. Genomics (1998) 52: 223-229; 46% identity in IGS4A amino acid residues 26-342).

Homology searches of DNA databanks with the IGS4 cDNA sequence yielded a number of entries which were also derived from the IGS4 gene locus (Fig.2 for overview):

- 10 EST sequence entries (accession nrs W61169, AI432384, W61131, AI023570, F01358, F03770, Z38158, R40869, R37725, H11333), 2 STS (sequence tagged sites) (accession nrs G20615 and G05725) and one genomic sequence (accession nr AQ078563) were discovered which were all derived from the 3'-UTR of IGS4 cDNA.
- EST accession n° N45474 encoded the 3' end of the IGS4 coding sequence and part of the 3' UTR (cfr supra).
- A 'working draft' high throughput genomic sequence (accession nr AC008571, version AC008571.1, deposited 3 AUG 1999), which consisted of 42 unordered contigs assembled in an arbitrary order was discovered in which we detected the entire IGS4 cDNA sequence in 4 separate areas. These areas most likely correspond to the different IGS4 exons as they were flanked by canonical splice donor and acceptor sequences. On the basis of this analysis the position of the different exons in the IGS4ADNA (or IGS4BDNA) sequence can be defined as follows: exon1 (1-780), exon 2 (781-865), exon 3 (866-991) and exon 4 (992-1658). The AC008571genomic sequence is of the IGS4A

allelic type.

- 6 overlapping EST entries (accession nrs H11359, R13890, R13353, F07531, F05108, F05107) were discovered of which the assembled DNA sequence overlapped at its 3' end with IGS4 exon2 and the beginning of exon 3. However the DNA sequence upstream of exon 2 was completely different from IGS4 exon1. Probably these six EST's are derived from transcripts which originated from an alternative promoter.
- Finally 2 genomic sequence entries (accession nrs AQ019411 and AQ015065) were discovered which encoded exon 2.

Among the many IGS4 cDNA clones that we isolated in the different experiments described above, we also discovered a number of clones that contained a 64 bp deletion (pos 866-929 in IGS4ADNA) (besides a number of clones derived from unspliced [or partially spliced] transcripts). So far we only discovered truncated transcripts of the polymorphic type A. We refer to this splice variant cDNA sequence as IGS4A-64DNA (SEQ ID NO: 9 and SEQ ID NO: 11). Since this deletion occurs exactly at the exon 2/exon 3 boundary and since the last 2 nucleotides of the deleted fragment are "AG", it is likely that this deletion represents an alternative splicing event in which the "AG" within exon 3 served as a splice acceptor. The IGS4A reading frame encoded by the splice variant is frameshifted beyond the deletion point. The encoded (truncated) protein of 296 amino acids is referred to as IGS4A-64PROT (SEQ ID NO: 10 and SEQ ID NO: 12). Hydropathy analysis of the IGS4A-64PROT sequence shows that this protein only contains 5 transmembrane domains (corresponding to TM domains 1-5 of IGS4APROT). This truncated receptor might have physiological relevance.

The bacterial strain harboring plasmid HNT2322 (containing the IGS4ADNA insert) was recloned after replating on LB agar plates containing 100 µg ampicillin/ml and deposited both in the Innogenetics N.V. strain list (ICCG4320) and at the "Centraalbureau voor Schimmelculturen (CBS)" in Baarn, The Netherlands (accession n° CBS102221). Plasmid DNA was prepared from the recloned isolate and the insert was resequenced and found to be identical to the IGS4ADNA sequence.

The bacterial strain harboring plasmid HNT2363 (containing the IGS4BDNA insert) was recloned after replating on LB agar plates containing 100 µg ampicillin/ml and deposited both in the Innogenetics N.V. strain list (ICCG4340) and at the "Centraalbureau voor Schimmelculturen (CBS)" in Baarn, The Netherlands (accession n° CBS102222). Plasmid DNA was prepared from the recloned isolate and the insert was resequenced and found to be identical to the IGS4BDNA sequence.

EXAMPLE 2. SPECIFIC CHANGES IN INTRACELLULAR CALCIUM CONCENTRATIONS INDUCED IN CHOG 16-IGS4 CELLS BY NEUROMEDIN U.

Example 2a. Experimental Procedures: Method and Materials.

A. Method and Materials for IGS-4 transfected CHOG 16-cells.

The following materials were used in the experiments: Vector containing IGS4-DNA sequence (IGS4-pcDNA3.1); SuperFect Transfection Reagent (Qiagen); Nut-Mix F12 (Gibco) with 10% FCS, 0.028mg/ml Gentamycin (Gibco); 0.22mg/ml Hygromycin (Gibco).

Materials used for clone selection: Nut-Mix F12 with 10% FCS; 0.028mg/ml Gentamycin; 0.22mg/ml Hygromycin and 0.55mg/ml Geneticin (Gibco).

The following method was applied: Transfection with SuperFect Transfection Reagent was carried out as described by the manufacturer (Qiagen). Cells were plated in 24-well plates to 50% confluence. Per well 0.6µg/µl plasmid-DNA with 1µl SuperFect Transfection Reagent was added. After 24 hours the medium was changed and transfected cell clones were selected by Geneticin-containing selection-medium. IGS4 expressing cell clones were characterized by RT-PCR and Northern Blot.

B. Method and Materials for FLIPR-Assay.

Cell Preparation:

For cell preparation the following materials were employed: plates: clear, flat-bottom, black well 96-well plates (Costar); Media: growth medium: Nut-Mix F-12 (HAM) with Glutamax (Gibco) supplemented with 10% fetal calf serum (Gibco); Incubator: 5% CO_2, 37°C (Nuaire).

The method was performed as follows: Cells were seeded 24 hours or 48 hours prior to the experiment into black wall microplates. The cell density was $0.8x10^{-4}$ cells/well for 48 hour incubation and $2.2x10^{-4}$ cells/well for 24 hour incubation. All steps were done under sterile conditions.

Dye loading:

In order to observe changes in intracellular calcium levels, cells must be 'loaded' with a calcium-sensitive fluorescent dye. This dye, called FLUO-4 (Molecular Probes) is excited at 488nm, and emits light in the 500-560nm range, only if a complex with calcium is formed. The dye was used at 4µM final concentration. Pluronic acid was added to increase dye solubility and dye uptake into the cells. Probenicid, an anion exchange protein inhibitor, was added to the dye medium to increase dye retention in the cells.

The following materials were used:

- 2mM dye stock: 1mg Fluo-4 (Molecular Probes) solubilized in 443µl low-water DMSO (Sigma). Aliquots stored at –20.

- 20% pluronic acid solution: 400mg pluronic acid (Sigma) solubilized in 2ml low-water DMSO (Sigma) at 37°C. Stored at room temperature.
- Dye/pluronic acid mixture: Immediately before use, equal volumes of the dye stock and 20% pluronic acid were mixed. The dye and pluronic acid had a final concentration of 1mM and 10%, respectively.
- Probenicid, 250mM stock solution: 710mg probenicid (Sigma) solubilized in 5ml 1N NaOH and mixed with 5ml Hank's BSS without phenol red (Gibco) supplemented with 20mM HEPES.
- Loading-Buffer: 10.5ml Hank's BSS without phenol red (Gibco) supplemented with 20mM HEPES, 105µl probenicid, 210µl 1M HEPES.
- Wash-Buffer: Hank's BSS without phenol red (Gibco) supplemented with 20mM HEPES (Gibco) and 2.5mM probenicid.

The method was worked as follows: The 2mM stock of dye was mixed with an equal volume of 20% (w/v) pluronic acid immediately before adding to the loading-Buffer. The growth-medium was aspirated out of the well without disturbing the confluent cell layer. 100µl loading medium was dispensed into each well using a Multidrop (Labsystems). Cell were incubated in a 5% CO_2, 37°C incubator for 30 minutes. In order to calculate the background fluorescence, some wells were not dye loaded. The background fluorescence in these wells results from autofluorescence of the cells. After dye loading, cell were washed three times with Wash-Buffer (automated Denley cell washer) to reduce the basal fluorescence to 20.000-25.000 counts above background. 100 l buffer was added and cell were incubated at 37°C till start of the experiment.

C. Preparation of compound plates.

The peptides were prepared at 3µM (3x the final concentration) for initial screening. For concentration response curves peptide-solutions were prepared in concentration ranges from 30µM to 100nM. All peptides were diluted in buffer containing 0.1% BSA (Sigma).

The following materials were used: Peptides: porcine Neuromedin U25, rat Neuromedin U-23, porcine Neuromedin U-8 (Bachem); Dilutionbuffer: Hank's BSS without phenol red (Gibco) supplemented with 20mM HEPES (Gibco) and 0.1% BSA (Sigma); plates: clear, flat-bottom, 96-well plates (Costar).

D. Assay.

The FLIPR setup parameters were set to 0.4 sec exposure length, filter 1, 50µl fluid addition, pipettor height at 125µl, Dispense Speed 40µl/sec without mixing.

Example 2b. Results.

To identify the endogenous ligand for the orphan G protein coupled receptor (GPCR) IGS4, IGS4 (both forms IGS4A and IGS4B) was stably transfected in Chinese Hamster Ovary (CHO) cells. Since the G protein coupling mechanism of IGS4 was unknown, a specific CHO-cell strain was used. These CHO-

cells stable express the G-protein G 16 (CHOG 16, Molecular Devices), which is known as "universal adapter" for GPCRs (Milligan G., Marshall F. and Rees S. (1996), Gα16 as a universal G protein adapter: implications for agonist screening strategies. *TIPS* 17: 235-237).

The resulting CHOG 16-IGS4 cells were functionally screened on a Fluorometric Imaging Plate Reader (FLIPR) to measure mobilisation of intracellular calcium in response to putative peptide ligands.

At the concentration of 10nM neuromedin U-23 induced a large, transient and robust calcium-response. In contrast, CHOG 16 cells and CHOG 16 cells expressing another, unrelated orphan GPCR, did not respond to neuromedin U-23. The results of these experiments with IGS4B are shown in Fig. 4.

Furthermore, the concentration dependence of IGS4 activation by porcine and rat neuromedin U isoforms were investigated (for both forms IGS4A and IGS4B). In the range of 10^{-6}-10^{-12} M porcine neuromedin U-25, rat neuromedin U-23, porcine neuromedin U-8 induced specific IGS4-mediated calcium mobilisation in the FLIPR assay. All three Neuromedin U isoforms tested caused the same maximal activation of IGS4B with LogE.C_{50} values of –10.09 ± 0.08 (neuromedin U-8, n=4; 80 pM), -10.61.± 0.08 (neuromedin U-23, n=10; 50 pm) and –9.14 ± 0.09 (neuromedin U-25, n=3; 1.2 nM). Thus, all three peptides cause potent activation of in particular IGS4B, suggesting that neuromedin U is the natural agonist for this receptor. The results of these experiments with IGS4B are shown in Fig. 3a (neuromedin U-8), Fig. 3b (neuromedin U-23) and Fig. 3c (neuromedin U-25).

For the IGS4A receptor somewhat lower affinities were found, but still showing that the neuromedin U peptides are good ligands for IGS4 receptors in general. The log EC_{50} values found for IGS4A were as follows; for neuromedin U-8: log EC_{50} = –9.3 ± 0.09 (n=1; 485 pM); for neuromedin U-23: log EC_{50} = –7.27 ± 0.16 (n=6; 53 nM); and for neuromedin U-25: log EC_{50} = –6.18 ± 0.14 (n=3; 658 nM).

The calcium mobilisation response seen following activation of IGS4 by neuromedin U suggests that this receptor is coupled to G proteins of the Gq/11 subfamily. In addition, basal levels of intracellular cAMP were not modulated by porcine neuromedin U-8 (1 and 10µM) in CHOG 16-IGS4 cells, suggesting that this receptor does not couple to G proteins of the Gs subfamilies (data not shown).

EXAMPLE 3. IGS4 HYBRIDIZATION ON HUMAN MULTIPLE TISSUE EXPRESSION ARRAY (MTE™)

Human IGS4A DNA (± 730 bp BamHI / HindIII insert from pGEMT-hIGS4A [ICCG #4320]) was radiolabelled via random primed incorporation of [$^{s}_{x}$-^{32}P]-dCTP to a specific activity of > 10^9 cpm/µg using the Prime-It II kit™ (Stratagene). The labeled probe was purified from free label via Sephadex G-50 chromatography, denatured for 5 min. at 95°C and added to the ExpressHyb hybridization solution at a final concentration of 1-1.5 x 10^6 cpm/ml. The human Multiple Tissue Expression (MTE™) Array (Clontech # 7775-1) was prehybridized and hybridized in ExpressHyb solution at 65°C for 30 min and overnight respectively according to the recommendations of the supplier.

The hybridized MTE™ array was washed 5 times for 20 min in 2 x SSC, 1% SDS at 65°C and then 2 times for 20 min at 55°C in 0.1 x SSC, 0.5% SDS. After the washes the array was autoradiographed via phosphorimaging (Cyclone Storage Phosphor System, Packard) (Fig.5). Hybridization data of the MTE™ array were analyzed quantitatively using the OptiQuant Image Analysis Software (Packard). Signal intensity of different spot positions containing RNA was corrected for the average background signal obtained from empty positions. The signal intensity obtained from the spot containing E. coli DNA was considered to represent a sample exhibiting no IGS4 expression. Samples with signal intensities below that of E. coli DNA were considered to be negative.

Hybridization signals for different tissues on the RNA array have been recalculated by subtracting each value with the hybridization signal observed for E. coli DNA (which is considered as the background signal). All tissues showing a lower hybridization signal are considered to be below background and to be IGS4 negative. Expression levels relative to that found in testis (100%) have been plotted and are provided in Fig.7.

EXAMPLE 4. TISSUE DISTRIBUTION OF IGS4 BY NORTHERN BLOT ANALYSIS

Human IGS4A DNA (± 730 bp BamHI / HindIII insert from pGEMT-hIGS4A [ICCG #4320]) was radiolabelled via random primed incorporation of [$^{s}\alpha$-^{32}P]-dCTP to a specific activity of > 10^9 cpm/µg using the Prime-It II kit™ (Stratagene). The labeled probe was purified from free label via Sephadex G-50 chromatography, denatured for 5 min. at 95°C and added to the ExpressHyb hybridization solution at a final concentration of 1-1.5 x 10^6 cpm/ml. The human Northern blots (Clontech #7760-1, #7759-1, #7767-1, #7755-1 and #7769-1) were prehybridized and hybridized in ExpressHyb solution at 65°C for 30 min and overnight respectively according to the recommendations of the supplier.

After hybridization Northern blots were washed 4 times 10 min at room temperature in 2 x SSC, 0.05 % SDS and then 2 times 40 min at 50°C in 0.1 x SSC, 0.1% SDS. After the washes the Northern blots were autoradiographed using phosphor storage plates (Cyclone Storage Phosphor System, Packard) and X-ray films. Results of Northern blots are shown in Fig.6.

The results of the Northern blot analysis appear to be largely consistent with those from the array hybridization (Example 3). The strongest signal (2.4 kb transcript) by far is found in testis. A weak 2.4 kb band was found in thymus, spinal cord, medulla, thyroid, thalamus, substantia nigra and a very weak band in corpus callosum, caudate nucleus and stomach. For some tissues no 2.4 kb band could be seen on Northern whereas a strong to moderate hybridization signal was observed on the MTE array (e.g. whole brain, cerebral cortex, lung, temporal and frontal lobe, amygdala, cerebellum, kidney and hippocampus).

EXAMPLE 5. QUANTITATIVE RT-PCR ANALYSIS.

IGS4 expression levels in different human tissues were also determined via real-time quantitative RT-PCR (Q-PCR) using the LightCycler™ instrument (Roche Diagnostics) and IGS4 specific TaqMan™ probes.

Example 5a. Experimental procedures.

A. cDNA synthesis.

Prior to reverse transcription 3 µg total RNA from the human total RNA panels I to V (Clontech # K4000-1 to K4004-1) was treated with 3U DNAse I (Life Technologies # 18068-015) in a 30 µl reaction volume (20 mM Tris pH 8.3, 50 mM KCl, 2 mM KCl) for 15 min at room temperature to destroy possibly contaminating genomic DNA. The reaction was stopped by adding 3 µl 25 mM EDTA and heating for 10 min at 65°C. 2,6 µg of the DNAse treated RNA was annealed with 1,3 µg oligo(dT) (Life Technologies # 18418-012) and subjected to reverse transcription using the Omniscript reverse transcriptase (Qiagen cat n° 205111) for 1 h at 37°C in a 52 µl reaction volume according to the protocol recommended by the supplier of the enzyme. The Omniscript reverse transcriptase was inactivated by heating at 93°C for 5 min.

B. Q-PCR.

Quantitative PCR reactions were carried out in a 20 µl reaction mixture, containing 1X TaqMan™ Universal PCR Mastermix (PE Applied Biosystems cat #4304437), 0.12 mg BSA/ml, 900 nM of IGS4 specific forward and reverse primers (IP14,963 and IP14,964), 250 nM of the IGS4 specific TaqMan™ probe (IP14,962) and either 1.6 µl (IGS4) or 0.16 µl (GAPDH: glyceraldehyde-3 phosphate dehydrogenase) of the cDNA synthesis reaction as template. To set up the IGS4 standard curve a dilution series (10^7 -10^1copies/ reaction) of IGS4 plasmid ICCG 4320 was used whereas for the GAPDH standard curve a dilution series of the human brain cDNA synthesis reaction (0.16 µl, 0.016 and 0.0016 µl) was used as template. The 1X TaqMan™ Universal PCR Mastermix contained AmpliTaq Gold™ DNA polymerase, AmpErase™ UNG (uracil-N-glycosylase), dNTPs with dUTP, passive reference and optimized buffer components. IGS4 specific primers and TaqMan probe were designed using the Primer Express™ software (PE Applied Biosystems). Quantitative PCR reactions for human GAPDH were carried out under identical conditions as described for IGS4 except that GAPDH specific primers and TaqMan™ probe were used from the TaqMan™ GAPDH control reagents kit (PE Applied Biosystems cat n° 402869; sequence information not available from PE Applied Biosystems).

PCR reactions were carried out in glass capillary cuvettes in the LightCycler™ instrument. After an initial incubation at 50°C for 2 min to allow the AmpErase™ UNG reaction to proceed and activation of the AmpliTaq Gold DNA polymerase (95°C for 10 min), reaction mixtures were subjected to 40 cycles of denaturation (15 sec at 95°C) and annealing/extension (1 min at either 60 °C [GAPDH] or at 68°C

[IGS4]). Quantification of experimental samples was carried out using the LightCycler Software version 3.0. A good linear relationship was obtained between the amount of IGS4 plasmid and the release of reporter dye within the range of 10-10^7 IGS4 plasmid copies. We also obtained a linear standard curve with the GAPDH TaqMan™ probe using the serially diluted brain cDNA. Relative GAPDH expression levels were in the range of 0.4 to 10.2 % of that observed in skeletal muscle, which of all tissues tested had the highest GAPDH expression level. Relative IGS4 expression levels were expressed as a proportion of the level detected in spinal cord, which had the highest IGS4 expression of all tissues tested (Fig.8). We also plotted relative IGS4 expression levels after normalization for expression of the GAPDH house keeping gene (Fig.8).

Example 5b. Results.

Q-PCR using an IGS4 specific TaqMan probe showed that highest expression levels (without normalization for GAPDH) were found in spinal cord. IGS4 expression levels in spinal cord amounted to 11,467 copies mRNA / ng pA RNA (assuming 100 % efficiency of the cDNA synthesis reaction and assuming that pA RNA constitutes 2% of total RNA). High IGS4 expression levels were also found in brain (41 % of spinal cord levels), skeletal muscle (37%), cerebellum (31%), testis (19%) and in lung (12%) and heart (11%). Lower levels were found in fetal brain (5%), trachea (4%), prostate (2%) and thyroid (1.4%). After normalization for GAPDH expression, the relative IGS4 expression pattern remained largely unchanged with the exception of skeletal muscle, where the relative expression level dropped to 2% of that in spinal cord. As it is not clear whether normalization for GAPDH is a valid procedure (GAPDH expression levels can be expected to vary more or less in different cell/ tissue types) we prefer to focus on the non-normalized relative expression levels.

These Q-PCR data seem to be in line with expression data from RNA array (Example 3) and Northern blot (Example 4) hybridization experiments in the sense that testis, spinal cord and brain appear to be among the most prominent expression sites. However Q-PCR analysis additionally shows important expression in a number of other tissues, such as skeletal muscle, cerebellum, lung and heart.

The ligand neuromedin U has been proposed to be a neuropeptide or neuromodulator, without the knowledge of the specific receptor (Domin J., Ghatei M. A., Chohan P. and Bloom S. R. (1987), Peptides 8: 779-784). Our investigation shows, that IGS4 is a novel member of the neuromedin U-receptor family being expressed in CNS and PNS regions, the gastrointestinal, immunological, genitourinary and cardiovascular system, skeletal muscle, thyroid, and lung.

Table 8: Overview of the PCR oligonucleotide primers and TaqMan probe used in the IGS4 Q-PCR reactions.

SEQ ID NO: 32	IP 14,963	5'-CCTCTTCAGCCTGGCGGTCTCTG-3'

SEQ ID NO: 33	IP 14,964	5'- GGAGGCGAAGCACACGGTCTCA-3'
SEQ ID NO: 34	IP 14,962	5'(FAM)-AGATGTGGCGCAACTACCCTTTCTTGTTCGGGCC-(TAMRA)3'

All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

The figures show:

Fig.1 Schematic representation of the relative positions of the different cDNA clones that were isolated to generate the consensus IGS4 cDNA sequence. 5' and 3' RACE primers that were used are also indicated (IGS4R# and IGS4F# respectively) as well as the position of EST accession n° N45474. Primer IGS4R6 was located within intron 1. Some clones (e.g. HNT2311, HNT2312 and HNT2253) were only partially sequenced (only the part that was sequenced is indicated). CONSENSUS A and CONSENSUS B denote the consensus sequence of IGS4 allelic types A and B respectively. The nucleotide that was identified at each of the 4 polymorphic positions is indicated (shaded boxes) for each clone. "S" indicates a sequence ambiguity in clones HNT2211 and HNT2212 and means either "C" or "T". The coding area of IGS4A and IGS4B consensus sequences is indicated with "***". As there were some remaining sequence ambiguities in the 5' end of the consensus sequence, the IGS4ADNA and IGS4BDNA sequences have only been taken from position 86 until the end

Fig.2 Schematic representation of the relative positions of different DNA database entries compared to the IGS4 cDNA sequence. The IGS4 cDNA sequence is indicated with the boxes (the position of the IGS4 coding sequences is indicated with the filled boxes). The relative position of IGS4 exons 1-4 is indicated above the IGS4 cDNA sequence ("=="). The parts of the genomic sequence AC008571 that encode exons 1->4 are indicated with AC008571a->d respectively. The position of these fragments within the AC008571 sequence are: AC008571a (13129-13908 of the reverse complement of AC008571), AC008571b (51676-51760 of AC008571), AC008571c (79978-80103 of the reverse complement of AC008571) and AC008571d (83060-83728 of the reverse complement of AC008571). G05725 and G20615 are STS (sequence tagged sequence) entries whereas F05107, F05108, F07531, R13353, R13890, H11359, N45474, W61169, AI432384, W61131, AI023570, F01358, F03770, Z38158, R40869, R37725, H11333 are EST entries. The parts of genomic clones AQ019411 and AQ015065 that contain IGS4 exon 2 are indicated with ":". The 5' part of EST sequences F05107, F05108, F07531, R13353, R13890 and H11359 which is totally different from the IGS4 cDNA sequence is indicated with "*". AQ078563 is a genomic clone.

Fig.3 : IGS4 receptor activation by different Neuromedin U isoforms. CHOG 16-IGS4B cells were cultured in 96-well plates overnight and loaded with Fluo-4AM. The receptor mediated Ca^{2+} changes were measured with FLIPR (Molecular Devices). Maxima of the fluorescence change detected by the CCD camera were normalised to 1 and are depicted as counts.
Fig. 3a: results for neuromedin U-8;
Fig. 3b: results for neuromedin U-23;
Fig. 3c: results for neuromedin U-25.

Fig.4 Neuromedin U-23 induced intracellular Ca^{2+} mobilisation in CHOG 16-cells expressing IGS4B. Application of 10nM Neuromedin U-23 to the cell lines CHOG 16-IGS4, CHOG 16 and CHOG 16 transfected with an other orphan GPCR. Cells were cultured in 96-well plates overnight and loaded with Fluo-4AM. Receptor mediated intracellular Ca^{2+} changes were measured with FLIPR (Molecular Devices), depicted in counts detected by the CCD camera.

Fig. 5 Human multiple tissue expression array using a human IGS4 probe.

Fig. 6 Northern blot analysis using an IGS4 probe.

Fig. 7 IGS4 expression analysis (MTE blot).

Fig. 8 Relative expression levels of IGS4 mRNA as compared to the expression observed in spinal cord. Both non-normalized and GAPDH-normalized expression levels are shown.

Original (for **SUBMISSION**) - printed on 25.09.2000 12:53:10 PM

0-1	**Form - PCT/RO/134 (EASY) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared using	PCT-EASY Version 2.90 (updated 15.12.1999)
0-2	**International Application No.**	PCT/EP00/09584
0-3	**Applicant's or agent's file reference**	SPW99.06

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	page	44
1-2	line	25>
1-3	**Identification of Deposit**	
1-3-1	Name of depositary institution	Centraalbureau voor Schimmelcultures
1-3-2	Address of depositary institution	Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, Netherlands
1-3-3	Date of deposit	24 September 1999 (24.09.1999)
1-3-4	Accession Number	CBS 102221
1-4	**Additional Indications**	none
1-5	**Designated States for Which Indications are Made**	all designated States
1-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	none
2	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
2-1	page	44
2-2	line	30>
2-3	**Identification of Deposit**	
2-3-1	Name of depositary institution	Centraalbureau voor Schimmelcultures
2-3-2	Address of depositary institution	Oosterstraat 1, Postbus 273, NL-3740 AG Baarn, Netherlands
2-3-3	Date of deposit	24 September 1999 (24.09.1999)
2-3-4	Accession Number	CBS 102222
2-4	**Additional Indications**	none
2-5	**Designated States for Which Indications are Made**	all designated States
2-6	**Separate Furnishing of Indications** These indications will be submitted to the International Bureau later	none

FOR RECEIVING OFFICE USE ONLY

0-4	**This form was received with the international application:** (yes or no)	27.09.2000

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0-4-1	Authorized officer	Cocky van Amstel

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0-5	**This form was received by the international Bureau on:**	
0-5-1	Authorized officer	

Claims

1. An isolated polynucleotide comprising a nucleotide sequence selected from the group consisting of:
   a) a nucleotide sequence encoding the IGS4 polypeptide according to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8.
   b) a nucleotide sequence encoding the polypeptide encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands, in particular a nucleotide sequence corresponding to the SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7.
   c) a nucleotide sequence having at least 80 % (preferably at least 90%) sequence identity over its entire length to the nucleotide sequence of (a) or (b);
   d) a nucleotide sequence which is complimentary to the nucleotide sequence of (a) or (b) or (c).

2. The polynucleotide of claim 1 wherein said polynucleotide comprises the nucleotide sequence contained in SEQ ID NO: 1 encoding the IGS4 polypeptide of SEQ ID NO: 2 or the nucleotide sequence contained in SEQ ID NO: 3 encoding the IGS4 polypeptide of SEQ ID NO: 4 or the nucleotide sequence contained in SEQ ID NO: 5 encoding the IGS4 polypeptide of SEQ ID NO: 6 or the nucleotide sequence contained in SEQ ID NO: 7 encoding the IGS4 polypeptide of SEQ ID NO: 8.

3. The polynucleotide of claim 1 wherein said polynucleotide comprises a nucleotide sequence that is at least 80% identical to that of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or to the sequence of the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands over its entire length.

4. The polynucleotide of claim 3 which is the polynucleotide of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 7 or the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands.

5. The polynucleotide of claim 1-4 which is DNA or RNA.

6. An isolated nucleotide sequence encoding an IGS4 neuromedin receptor protein, preferably a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25.

7. An isolated nucleotide sequence of claim 6 encoding an IGS4 neuromedin receptor protein, said protein exhibiting expression in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea.

8. An isolated nucleotide sequence encoding an IGS4 neuromedin receptor protein, preferably a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, said protein exhibiting expression in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea, and said nucleotide sequence being selected from the group of nucleotide sequences as defined in the claims 1 to 5.

9. A DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing an IGS4 polypeptide comprising an amino acid sequence, which has at least 80% identity with the polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6 or SEQ ID NO: 8 or with the polypeptide encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands, when said expression system is present in a compatible host cell.

10. An isolated DNA or RNA molecule comprising an expression system, wherein said expression system is capable of producing an IGS4 polypeptide comprising an amino acid sequence which is a neuromedin receptor protein, preferably a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, and exhibiting expression in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus

accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea.

11. A host cell comprising the expression system of claim 9 or 10.

12. A host cell according to claim 11 which is a yeast cell.

13. A host cell according to claim 11 which is an animal cell.

14. IGS4 receptor membrane preparation derived from a cell according to claim 11-13.

15. A process for producing an IGS4 polypeptide comprising culturing a host of claim 11-13 under conditions sufficient for the production of said polypeptide and recovering the polypeptide from the culture.

16. A process for producing a cell which produces an IGS4 polypeptide comprising transforming or transfecting a host cell with the expression system of claim 9 or 10 such that the host cell, under appropriate culture conditions, produces an IGS4 polypeptide.

17. An IGS4 polypeptide comprising an amino acid sequence which is at least 80% identical to the amino acid sequence of SEQ ID NO: 2, SEQ NO: 4, SEQ NO: 6 or SEQ NO: 8 or to the polypeptide encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands over its entire length.

18. The polypeptide of claim 17 which comprises the amino acid sequence of SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 6 or SEQ NO: 8 or the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands.

19. An isolated IGS4 polypeptide comprising an amino acid sequence of a neuromedin receptor protein, preferably of a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25.

20. An isolated IGS4 polypeptide of claim 19 comprising an amino acid sequence of a neuromedin receptor protein, said protein exhibiting expression in brain, skeletal muscle,

cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea.

21. An isolated IGS4 polypeptide comprising an amino acid sequence of a neuromedin receptor protein, preferably a mammalian neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, said protein exhibiting expression in brain, skeletal muscle, cerebellum, testis, corpus callosum, spinal cord, substantia nigra, medulla, thalamus, caudate nucleus, pons, nucleus accumbens, fetal brain, stomach, heart, thyroid gland, lung, thymus, prostate and/or in trachea, and said amino acid sequence being selected from the group of amino acid sequence as defined in the claims 17-18.

22. An antibody immunospecific for the IGS4 polypeptide of claim 17-21.

23. A method for the treatment of a subject in need of enhanced activity or expression of IGS4 polypeptide of claim 17-21 comprising:

(a) administering to the subject a therapeutically effective amount of an agonist to said receptor; and/or

(b) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that has at least 80% identity to a nucleotide sequence encoding the IGS4 polypeptide of SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 6 or SEQ NO: 8 or the polypeptide encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands over its entire length; or a nucleotide sequence complementary to one of said nucleotide sequences in a form so as to effect production of said receptor activity in vivo.

(c) providing to the subject an isolated polynucleotide comprising a nucleotide sequence that encodes an IGS4 neuromedin receptor protein, preferably a mammalian IGS4 neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25.

24. A method for the treatment of a subject having need to inhibit activity or expression of IGS4 polypeptide of claim 17-21 comprising:

(a) administering to the subject a therapeutically effective amount of an antagonist to said receptor; and/or

(b) administering to the subject a nucleic acid molecule that inhibits the expression of the nucleotide sequence encoding said receptor; and/or

(c) administering to the subject a therapeutically effective amount of a polypeptide that competes with said receptor for its ligand.

25. A process for diagnosing a disease or a susceptibility to a disease in a subject related to expression or activity of the IGS4 polypeptide of claim 17-21 in a subject comprising:

(a) determining the presence or absence of a mutation in the nucleotide sequence encoding said IGS4 polypeptide in the genome of said subject; and/or

(b) analyzing for the presence or amount of the IGS4 polypeptide expression in a sample derived from said subject.

26. A method for identifying agonists to the IGS4 polypeptide of claim 17-21 comprising:

(a) contacting a cell which produces a IGS4 polypeptide with a test compound; and

(b) determining whether the test compound effects a signal generated by activation of the IGS4 polypeptide.

27. An agonist identified by the method of claim 26.

28. A method for identifying agonists to the IGS4 neuromedin receptor protein, preferably to the mammalian IGS4 neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, comprising:

(a) contacting a cell which produces a IGS4 neuromedin receptor protein with a test compound; and

(b) determining whether the test compound effects a signal generated by activation of the IGS4 neuromedin receptor protein.

29. A method for identifying agonists to the IGS4 neuromedin receptor protein according to claim 28, wherein said agonists are effective with regard to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

30. An agonist identified by the method of claim 28 or 29, preferably an agonist being effective with regard to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

31. A method for identifying antagonists to the IGS4 polypeptide of claim 17-21 comprising:

(a) contacting a cell which produces a IGS4 polypeptide with an agonist; and

(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

32. An antagonist identified by the method of claim 31.

33. A method for identifying antagonists to the IGS4 neuromedin receptor protein, preferably to the mammalian IGS4 neuromedin receptor protein, said protein exhibiting high affinity binding for neuromedin U, preferably for neuromedin U-8, for neuromedin U-23 and/or for neuromedin U-25, comprising:

(a) contacting a cell which produces a IGS4 neuromedin receptor protein with an agonist; and

(b) determining whether the signal generated by said agonist is diminished in the presence of a candidate compound.

34. A method for identifying antagonists to the IGS4 neuromedin receptor protein according to claim 33, wherein said antagonists are effective with regard to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

35. An antagonist identified by the method of claim 33 or 34, preferably an antagonist being effective with regard to disorders of the nervous system, including the central nervous system (CNS) and the peripheral nervous system (PNS), disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system.

36. A recombinant host cell produced by a method of claim 16 or a membrane thereof expressing an IGS4 polypeptide.

37. A method of creating a genetically modified non-human animal comprising the steps of:

(a) ligating the coding portion of a nucleic acid molecule, consisting essentially of a nucleic acid sequence encoding a protein having the amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 6 or SEQ NO: 8 or the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands or a biologically active portion of one of said sequences, to a regulatory sequence which is capable of driving high level gene expression or expression in a cell type in which the gene is not normally expressed in said animal; or

(b) isolation and engineering the coding portion of a nucleic acid molecule, consisting essentially of a nucleic acid sequence encoding a protein having the amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 6 or SEQ NO: 8 or the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands or a biologically active portion of one of said sequences, and reintroducing said sequence in the genome of said animal in such a way that the endogenous gene alleles, encoding a protein having the amino acid sequence SEQ ID NO: 2, SEQ ID NO: 4, SEQ NO: 6 or SEQ NO: 8 or the amino acid sequence encoded by the DNA insert contained in the deposit no. CBS102221 or the deposit no. CBS102222 at the Centraalbureau voor Schimmelcultures at Baarn the Netherlands or a biologically active portion of one of said sequences, are fully or partially inactivated.

38. A method of determining whether a substance is a potential ligand of IGS4 receptor comprising:

(a) contacting cells expressing the receptor of one of the claims 17-21 or one of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8, or contacting a receptor membrane preparation comprising one of said receptors of one of the claims 17-21 or one of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6 and SEQ ID NO:8 with labeled neuromedin U in the presence and in the absence of the substance; and

(b) measuring the binding of neuromedin U to IGS4.

39. A polypeptide according to any of the claims 17-21, further being characterized in that said polypeptide binds neuromedin U, preferably neuromedin U-8, neuromedin U-23 and/or neuromedin U-25, showing at least an affinity of about log EC_{50}=-6.

40. A polypeptide according to any of the claims 17-21, further being characterized in that said polypeptide binds neuromedin U, preferably neuromedin U-8, neuromedin U-23 and/or neuromedin U-25, showing at least an affinity of about log EC_{50}=-9.

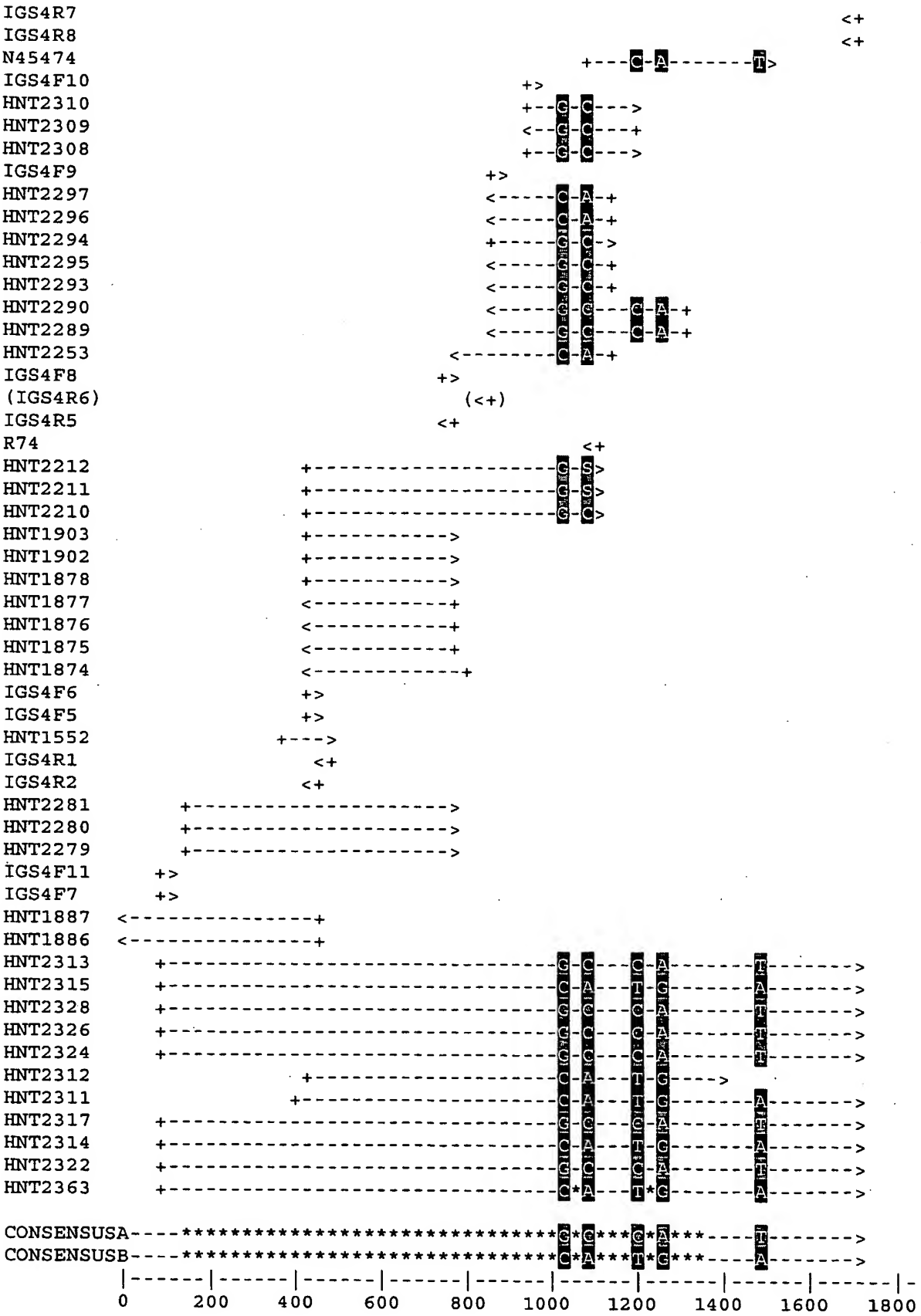
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Fig.1

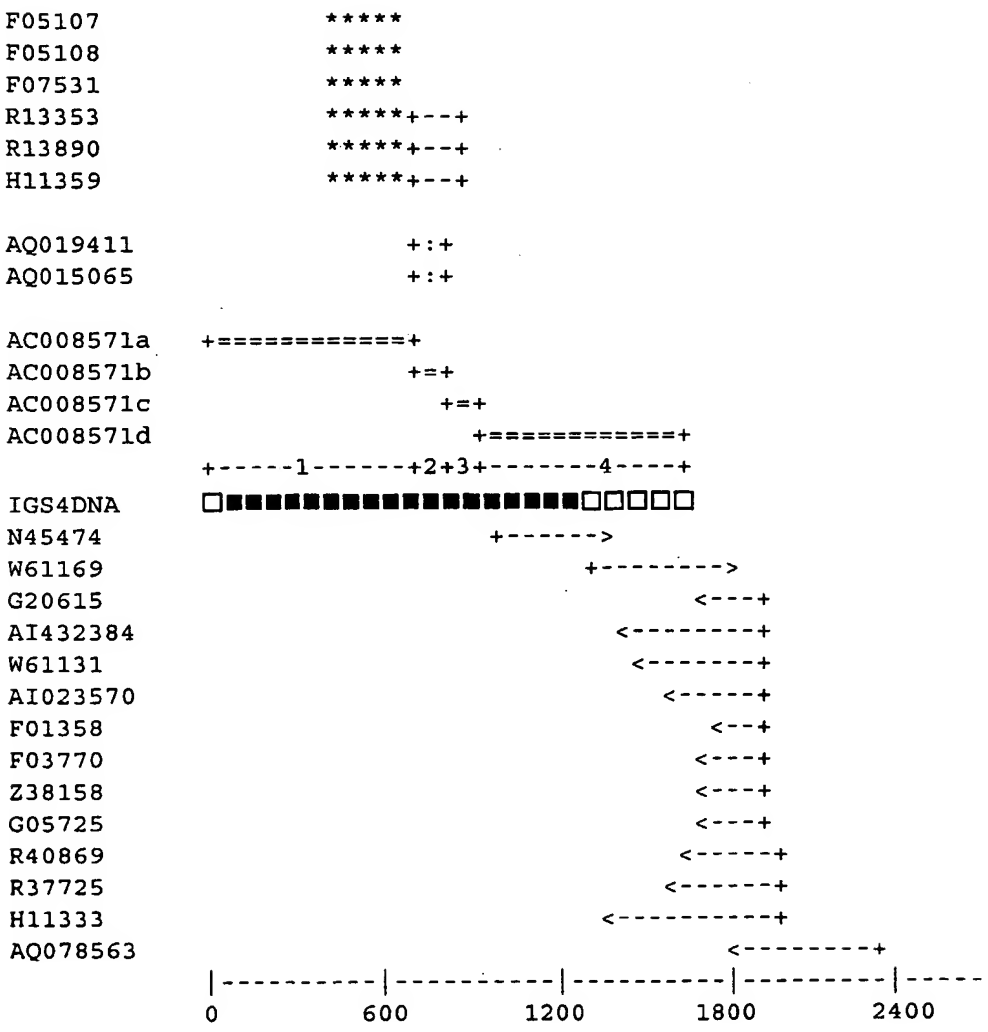
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Fig.2

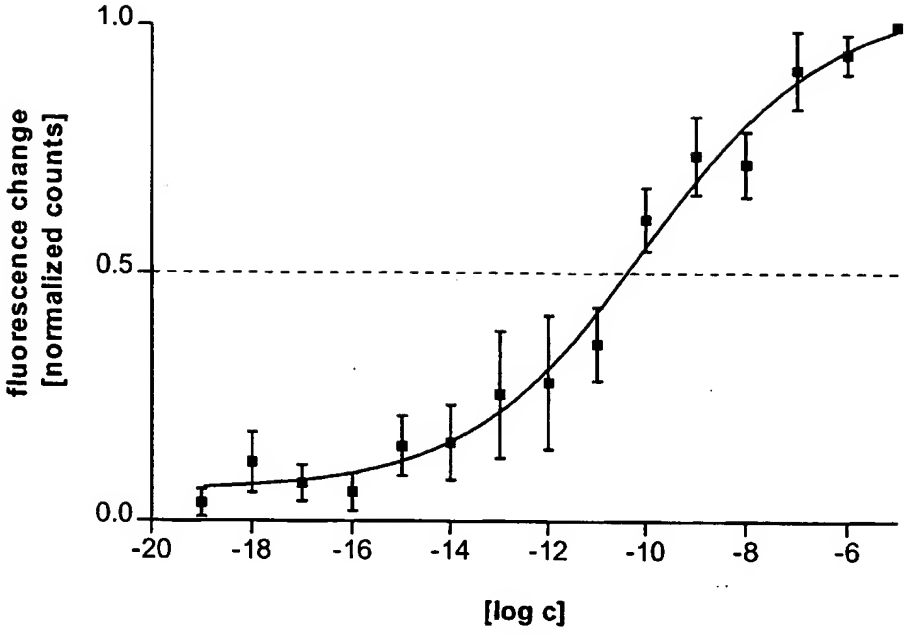
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Fig. 3a

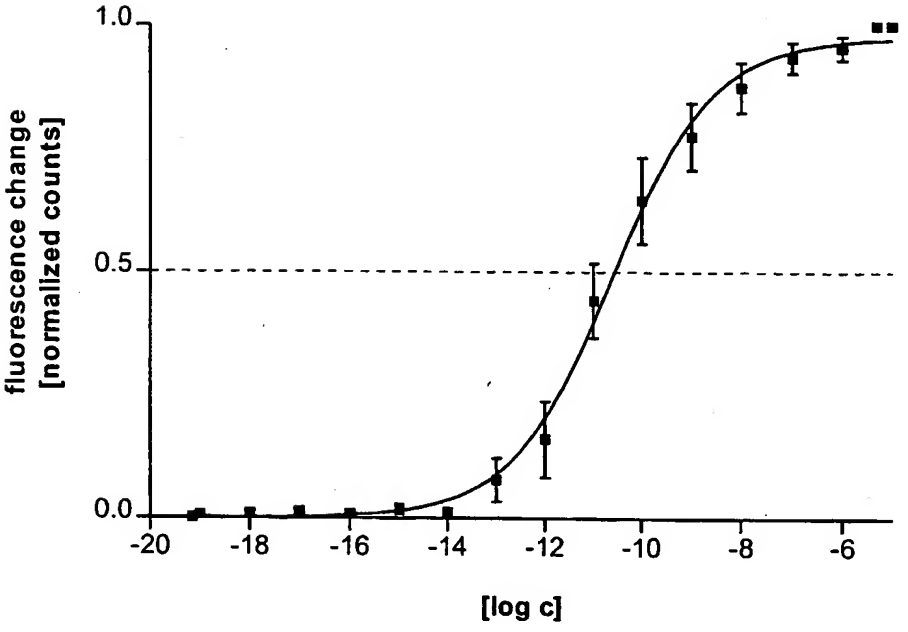
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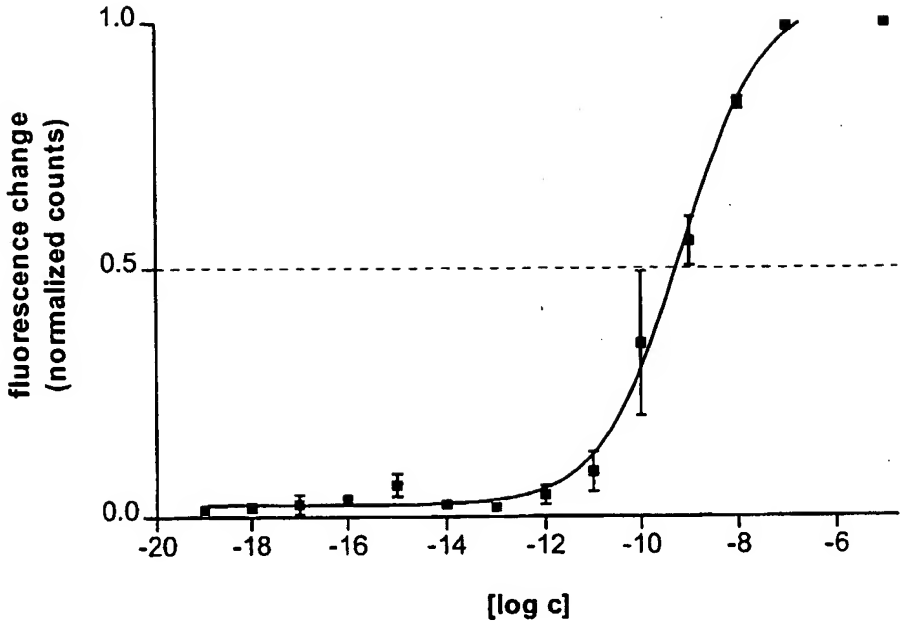
Fig. 3b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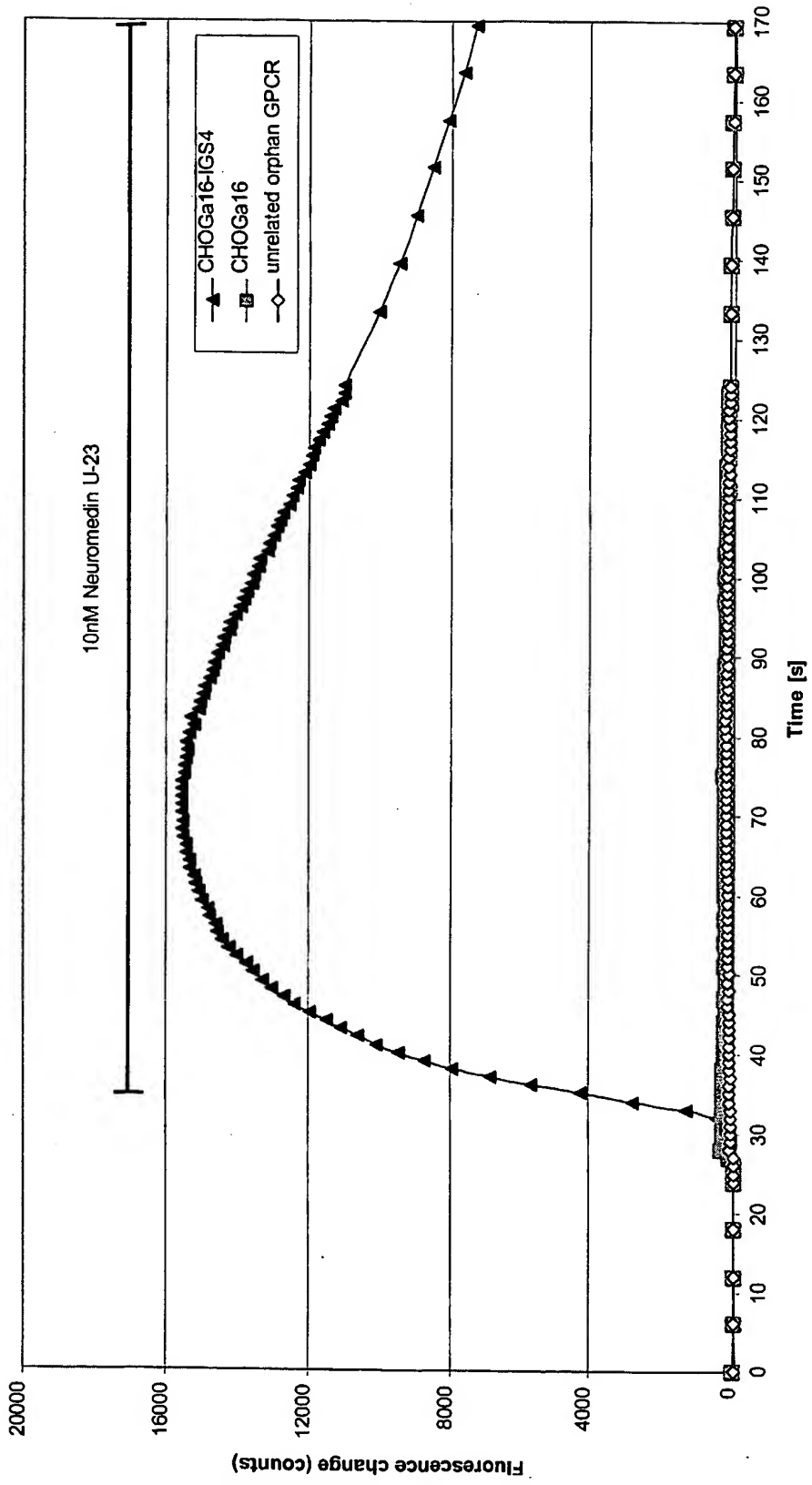
[log c]

Fig. 3c

Fig. 4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CHOGa16
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Fluorescence change (counts)
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20000
0
10
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Fig. 5

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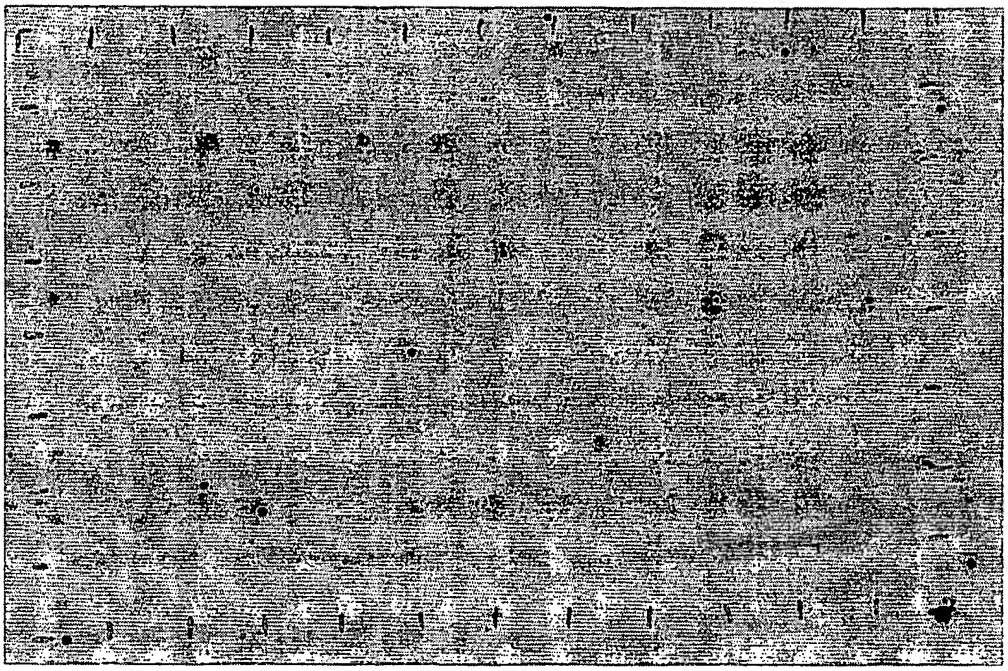

2. H1 H12

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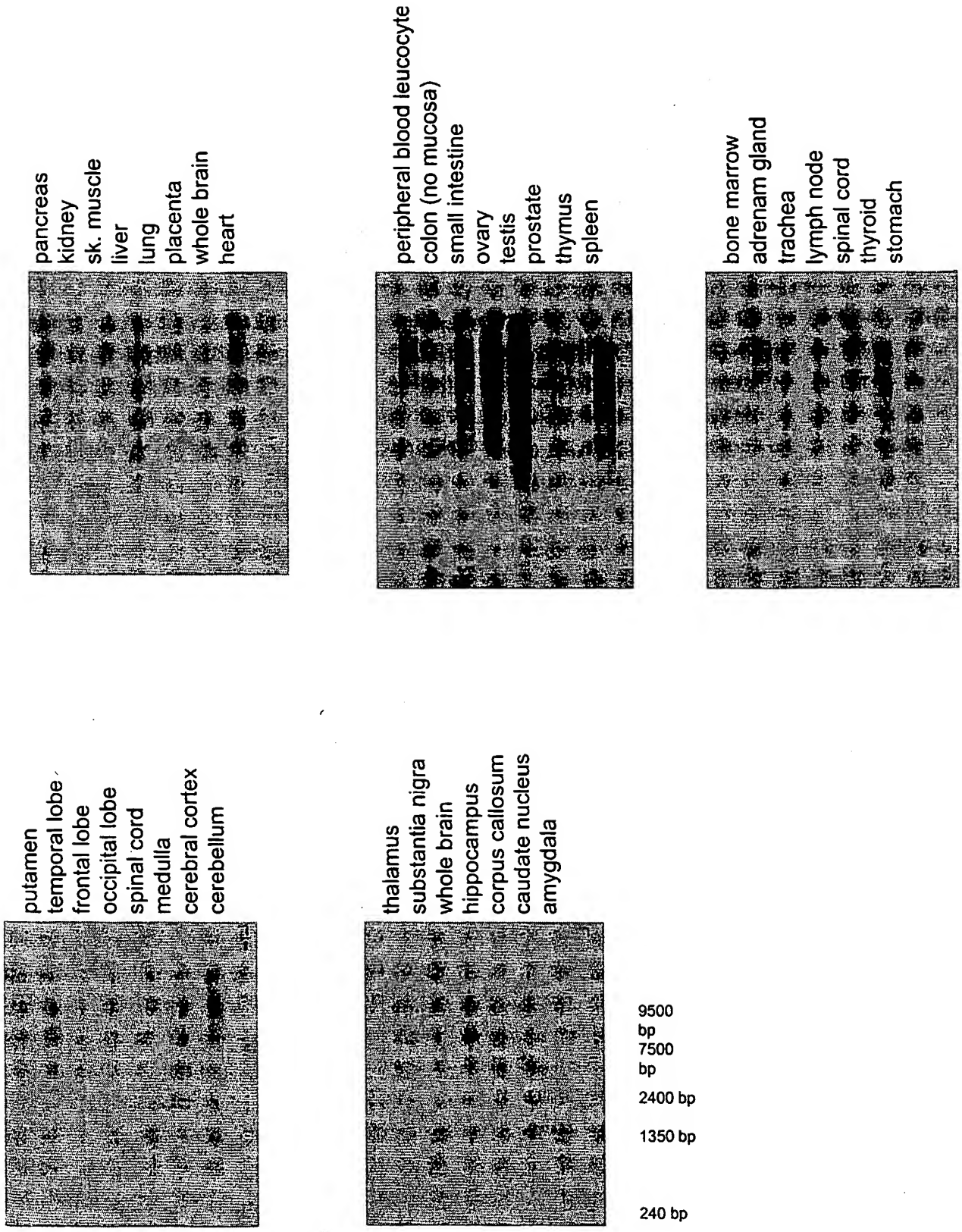
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liver
lung
placenta
whole brain
heart
peripheral blood leucocyte
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small intestine
ovary
testis
prostate
thymus
spleen
bone marrow
adrenam gland
trachea
lymph node
spinal cord
thyroid
stomach
putamen
temporal lobe
frontal lobe
occipital lobe
spinal cord
medulla
cerebral cortex
cerebellum
thalamus
substantia nigra
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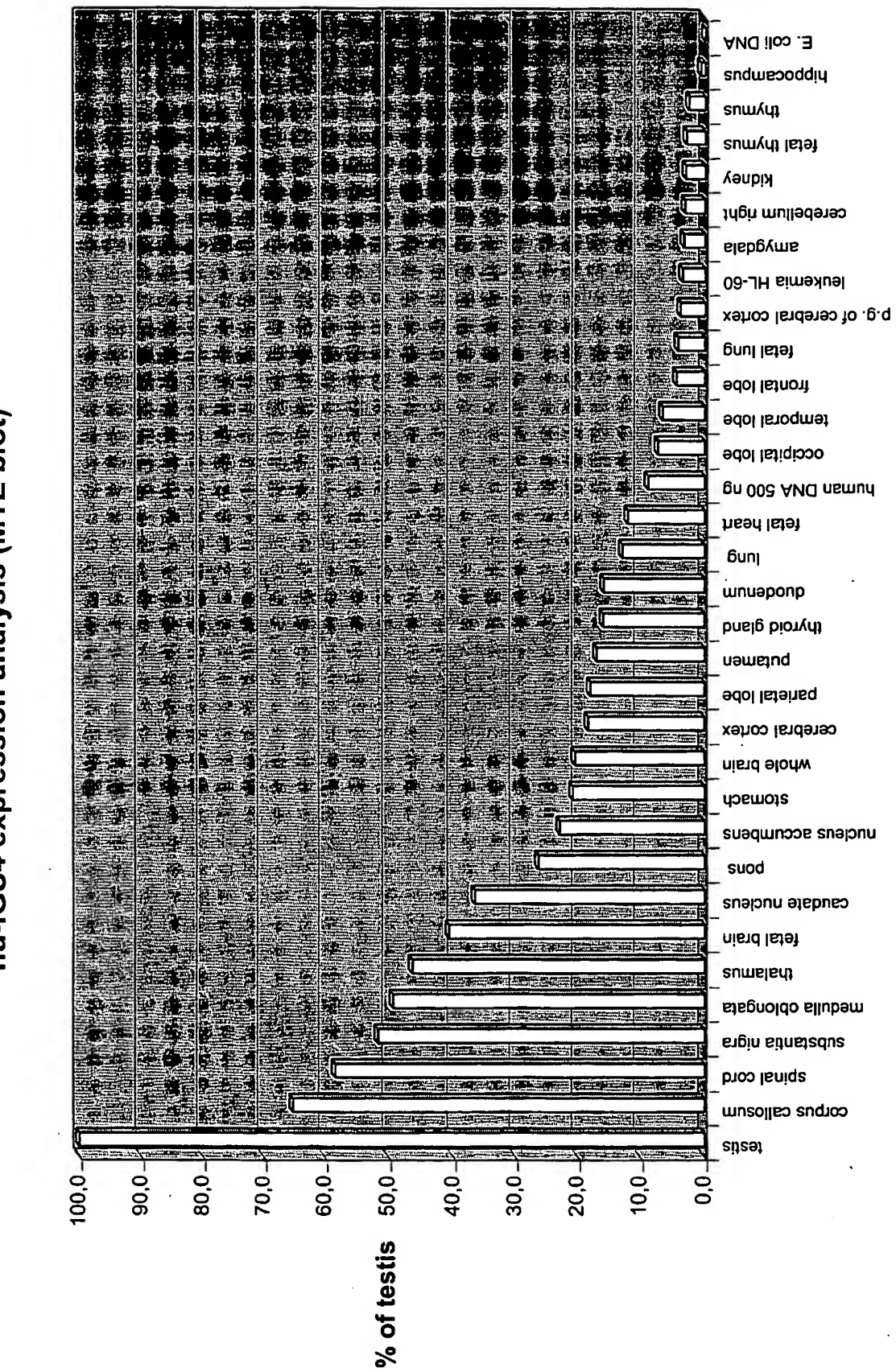
Fig. 6

Fig. 7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10,0
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corpus callosum
spinal cord
substantia nigra
medulla oblongata
thalamus
fetal brain
caudate nucleus
pons
nucleus accumbens
stomach
whole brain
cerebral cortex
parietal lobe
putamen
thyroid gland
duodenum
lung
fetal heart
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occipital lobe
temporal lobe
frontal lobe
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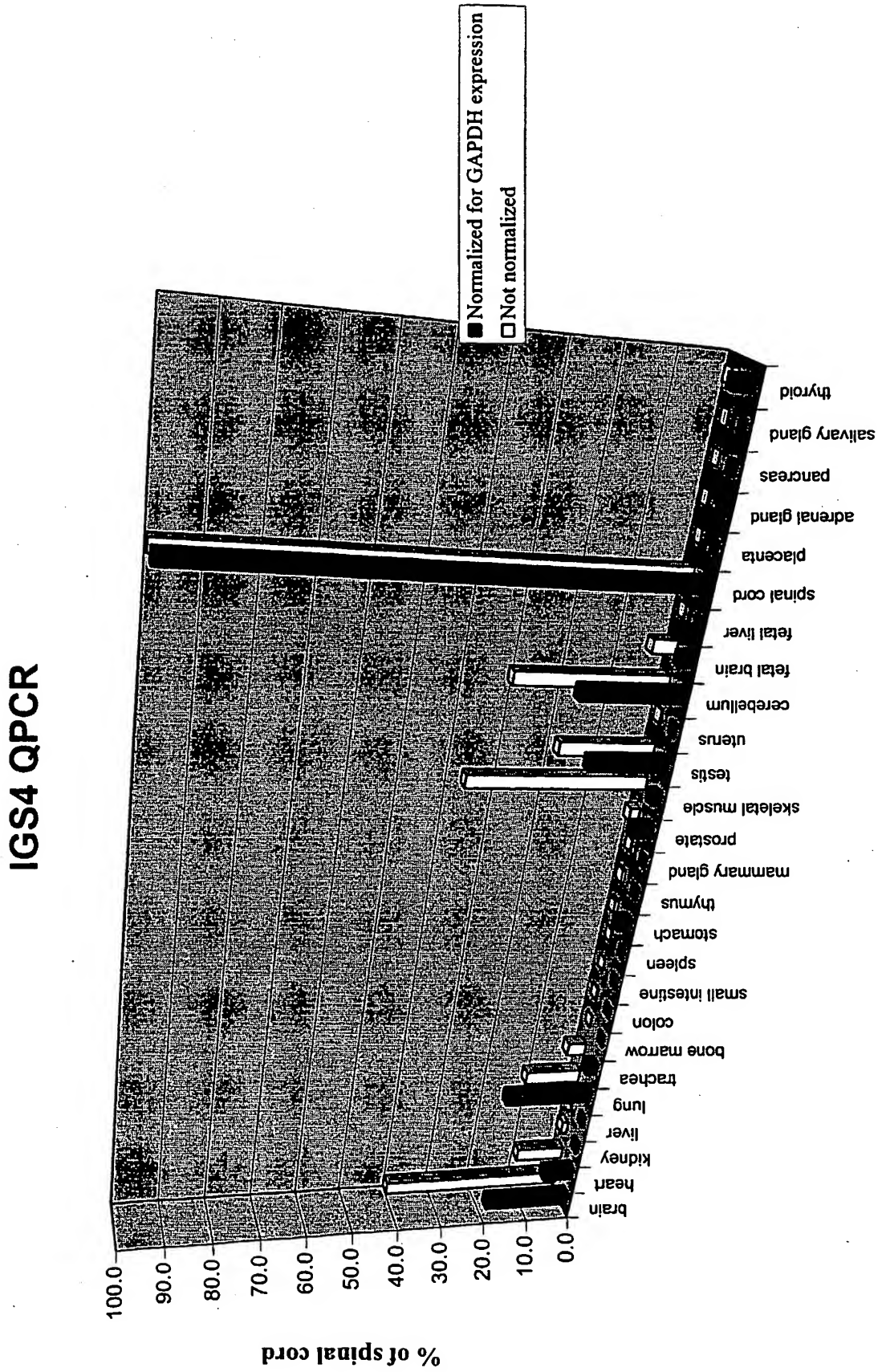
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Not normalized
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20.0
10.0
0.0
brain
heart
kidney
liver
lung
trachea
bone marrow
colon
small intestine
spleen
stomach
thymus
mammary gland
prostate
skeletal muscle
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uterus
cerebellum
fetal brain
fetal liver
spinal cord
placenta
adrenal gland
pancreas
salivary gland
thyroid

Fig. 8

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5 10 15

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70 75 80

tac tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt 345
Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu
85 90 95

gga atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg 393
Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu

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        100                 105                 110

ttc ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg   441
Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val
    115                 120                 125

tgc ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac   489
Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr
130                 135                 140                 145

gtg gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc   537
Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg
                150                 155                 160

cgg gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc   585
Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe
            165                 170                 175

tcc ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc   633
Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro
        180                 185                 190

aat ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc   681
Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro
    195                 200                 205

atg tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac   729
Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr
210                 215                 220                 225

ctc ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc   777
Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu
                230                 235                 240

aga cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat   825
Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn
            245                 250                 255

att caa aga ccc tgc aga aaa tca gtc aac aag atg ctg ttt gtc ttg   873
Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu
        260                 265                 270

gtc tta gtg ttt gct atc tgt tgg gcc ccg ttc cac att gac cga ctc   921
Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu
    275                 280                 285

ttc ttc agc ttt gtg gag gag tgg agt gaa tcc ctg gct gct gtg ttc   969
Phe Phe Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val Phe
290                 295                 300                 305

aac ctc gtc cat gtg gtg tca ggt gtc ttc ttc tac ctg agc tca gct  1017
Asn Leu Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser Ala
                310                 315                 320

gtc aac ccc att atc tat aac cta ctg tct cgc cgc ttc cag gca gca  1065
Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala
```

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            325                 330                 335

ttc cag aat gtg atc tct tct ttc cac aaa cag tgg cac tcc cag cat   1113
Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His
        340                 345                 350

gac cca cag ttg cca cct gcc cag cgg aac atc ttc ctg aca gaa tgc   1161
Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys
    355                 360                 365

cac ttt gtg gag ctg acc gaa gat ata ggt ccc caa ttc cca tgt cag   1209
His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys Gln
370                 375                 380                 385

tca tcc atg cac aac tct cac ctc cca aca gcc ctc tct agt gaa cag   1257
Ser Ser Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln
                390                 395                 400

atg tca aga aca aac tat caa agc ttc cac ttt aac aaa acc           1299
Met Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
            405                 410                 415

tgaattcttt cagagctgac tctcctctat gcctcaaaac ttcagagagg aacatcccat 1359

aatgtatgcc ttctcatatg atattagaga ggtagaatgg ctcttacaac tcatgtaccc 1419

attgctagtt ttttttttt aataaacgtg aaaactgaga gttagatctg gtttcaaaac 1479

ccaagactgc ctgattttta gttatctttc cactatccta actgcctcat gccccttcac 1539

tagttcatgc caagaacgtg actggaaagg catggcacct ataccttgat taatttccat 1599

taatggaaat ggttcgtcct gagtcatcta cgttccgagt caggctgtca ctcctacta  1658
```

<210> 2
<211> 415
<212> PRT
<213> Homo sapiens

<400> 2

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Met Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln
  1               5                  10                  15

Lys Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr
             20                  25                  30

Leu Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val
         35                  40                  45

Ser Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val
     50                  55                  60

Leu Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr
 65                  70                  75                  80
```

```
Asn Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu
                 85                  90                  95

Leu Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe
            100                 105                 110

Leu Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr
        115                 120                 125

Val Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg
    130                 135                 140

Tyr Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg
145                 150                 155                 160

Arg Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu
                165                 170                 175

Phe Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe
            180                 185                 190

Pro Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys
        195                 200                 205

Pro Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe
    210                 215                 220

Tyr Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala
225                 230                 235                 240

Leu Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala
                245                 250                 255

Asn Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val
            260                 265                 270

Leu Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg
        275                 280                 285

Leu Phe Phe Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val
    290                 295                 300

Phe Asn Leu Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser
305                 310                 315                 320

Ala Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala
                325                 330                 335

Ala Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln
            340                 345                 350

His Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu
        355                 360                 365

Cys His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys
    370                 375                 380
```

```
Gln Ser Ser Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu
385                 390                 395                 400

Gln Met Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
                405                 410                 415



<210> 3
<211> 1658
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (64)..(1299)
<223> IGS4A short version

<400> 3
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat tttaatgtca 60

ggg atg gaa aaa ctt cag aat gct tcc tgg atc tac cag cag aaa cta   108
    Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu
      1               5                  10                  15

gaa gat cca ttc cag aaa cac ctg aac agc acc gag gag tat ctg gcc   156
Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala
                 20                  25                  30

ttc ctc tgc gga cct cgg cgc agc cac ttc ttc ctc ccc gtg tct gtg   204
Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val
             35                  40                  45

gtg tat gtg cca att ttt gtg gtg ggg gtc att ggc aat gtc ctg gtg   252
Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val
         50                  55                  60

tgc ctg gtg att ctg cag cac cag gct atg aag acg ccc acc aac tac   300
Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr
     65                  70                  75

tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt gga   348
Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu Gly
 80                  85                  90                  95

atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg ttc   396
Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe
                100                 105                 110

ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg tgc   444
Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys
            115                 120                 125

ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac gtg   492
Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val
```

```
        130                 135                 140

gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc cgg   540
Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg
    145                 150                 155

gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc tcc   588
Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser
160                 165                 170                 175

ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc aat   636
Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn
                180                 185                 190

ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc atg   684
Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met
            195                 200                 205

tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac ctc   732
Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu
        210                 215                 220

ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc aga   780
Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg
    225                 230                 235

cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat att   828
Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile
240                 245                 250                 255

caa aga ccc tgc aga aaa tca gtc aac aag atg ctg ttt gtc ttg gtc   876
Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu Val
                260                 265                 270

tta gtg ttt gct atc tgt tgg gcc ccg ttc cac att gac cga ctc ttc   924
Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu Phe
            275                 280                 285

ttc agc ttt gtg gag gag tgg agt gaa tcc ctg gct gct gtg ttc aac   972
Phe Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val Phe Asn
        290                 295                 300

ctc gtc cat gtg gtg tca ggt gtc ttc ttc tac ctg agc tca gct gtc   1020
Leu Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser Ala Val
    305                 310                 315

aac ccc att atc tat aac cta ctg tct cgc cgc ttc cag gca gca ttc   1068
Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala Phe
320                 325                 330                 335

cag aat gtg atc tct tct ttc cac aaa cag tgg cac tcc cag cat gac   1116
Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His Asp
                340                 345                 350

cca cag ttg cca cct gcc cag cgg aac atc ttc ctg aca gaa tgc cac   1164
Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys His
```

```
            355                 360                 365

ttt gtg gag ctg acc gaa gat ata ggt ccc caa ttc cca tgt cag tca   1212
Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys Gln Ser
        370                 375                 380

tcc atg cac aac tct cac ctc cca aca gcc ctc tct agt gaa cag atg   1260
Ser Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln Met
    385                 390                 395

tca aga aca aac tat caa agc ttc cac ttt aac aaa acc tgaattcttt    1309
Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
400                 405                 410

cagagctgac tctcctctat gcctcaaaac ttcagagagg aacatcccat aatgtatgcc 1369

ttctcatatg atattagaga ggtagaatgg ctcttacaac tcatgtaccc attgctagtt 1429

ttttttttt aataaacgtg aaaactgaga gttagatctg gtttcaaaac ccaagactgc 1489

ctgattttta gttatctttc cactatccta actgcctcat gccccttcac tagttcatgc 1549

caagaacgtg actggaaagg catggcacct ataccttgat taatttccat taatggaaat 1609

ggttcgtcct gagtcatcta cgttccgagt caggctgtca ctcctacta             1658


<210> 4
<211> 412
<212> PRT
<213> Homo sapiens

<400> 4
Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu Glu
  1               5                  10                  15

Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala Phe
             20                  25                  30

Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val Val
         35                  40                  45

Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val Cys
     50                  55                  60

Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr Tyr
 65                  70                  75                  80

Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Gly Met
                 85                  90                  95

Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe Gly
            100                 105                 110

Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys Phe
        115                 120                 125
```

```
Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val Ala
    130                 135                 140

Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg Ala
145                 150                 155                 160

Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser Leu
                165                 170                 175

Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn Gly
            180                 185                 190

Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met Trp
        195                 200                 205

Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu Leu
    210                 215                 220

Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg Leu
225                 230                 235                 240

Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile Gln
                245                 250                 255

Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu Val Leu
            260                 265                 270

Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu Phe Phe
        275                 280                 285

Ser Phe Val Glu Glu Trp Ser Glu Ser Leu Ala Ala Val Phe Asn Leu
    290                 295                 300

Val His Val Val Ser Gly Val Phe Phe Tyr Leu Ser Ser Ala Val Asn
305                 310                 315                 320

Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala Phe Gln
                325                 330                 335

Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His Asp Pro
            340                 345                 350

Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys His Phe
        355                 360                 365

Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Pro Cys Gln Ser Ser
    370                 375                 380

Met His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln Met Ser
385                 390                 395                 400

Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
                405                 410
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<210> 5
<211> 1658
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (55)..(1299)
<223> IGS4B long version

<400> 5
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat ttta atg 57
Met
1

tca ggg atg gaa aaa ctt cag aat gct tcc tgg atc tac cag cag aaa 105
Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys
5 10 15

cta gaa gat cca ttc cag aaa cac ctg aac agc acc gag gag tat ctg 153
Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu
20 25 30

gcc ttc ctc tgc gga cct cgg cgc agc cac ttc ttc ctc ccc gtg tct 201
Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser
35 40 45

gtg gtg tat gtg cca att ttt gtg gtg ggg gtc att ggc aat gtc ctg 249
Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu
50 55 60 65

gtg tgc ctg gtg att ctg cag cac cag gct atg aag acg ccc acc aac 297
Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn
70 75 80

tac tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt 345
Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu
85 90 95

gga atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg 393
Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu
100 105 110

ttc ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg 441
Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val
115 120 125

tgc ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac 489
Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr
130 135 140 145

gtg gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc 537
Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg
150 155 160

```
cgg gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc   585
Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe
            165                 170                 175

tcc ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc   633
Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro
        180                 185                 190

aat ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc   681
Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro
    195                 200                 205

atg tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac   729
Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr
210                 215                 220                 225

ctc ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc   777
Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu
                230                 235                 240

aga cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat   825
Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn
            245                 250                 255

att caa aga ccc tgc aga aaa tca gtc aac aag atg ctg ttt gtc ttg   873
Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu
        260                 265                 270

gtc tta gtg ttt gct atc tgt tgg gcc ccg ttc cac att gac cga ctc   921
Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu
    275                 280                 285

ttc ttc agc ttt gtg gag gag tgg act gaa tcc ctg gct gct gtg ttc   969
Phe Phe Ser Phe Val Glu Glu Trp Thr Glu Ser Leu Ala Ala Val Phe
290                 295                 300                 305

aac ctc gtc cat gtg gtg tca ggt gtc tta ttc tac ctg agc tca gct   1017
Asn Leu Val His Val Val Ser Gly Val Leu Phe Tyr Leu Ser Ser Ala
                310                 315                 320

gtc aac ccc att atc tat aac cta ctg tct cgc cgc ttc cag gca gca   1065
Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala
            325                 330                 335

ttc cag aat gtg atc tct tct ttc cac aaa cag tgg cac tcc cag cat   1113
Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His
        340                 345                 350

gac cca cag ttg cca cct gcc cag cgg aac atc ttc ctg aca gaa tgc   1161
Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys
    355                 360                 365

cac ttt gtg gag ctg acc gaa gat ata ggt ccc caa ttc cta tgt cag   1209
His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Leu Cys Gln
370                 375                 380                 385
```

```
tca tcc gtg cac aac tct cac ctc cca aca gcc ctc tct agt gaa cag   1257
Ser Ser Val His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln
                390                 395                 400

atg tca aga aca aac tat caa agc ttc cac ttt aac aaa acc           1299
Met Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
            405                 410                 415

tgaattcttt cagagctgac tctcctctat gcctcaaaac ttcagagagg aacatcccat 1359

aatgtatgcc ttctcatatg aaattagaga ggtagaatgg ctcttacaac tcatgtaccc 1419

attgctagtt ttttttttt aataaacgtg aaaactgaga gttagatctg gtttcaaaac 1479

ccaagactgc ctgattttta gttatctttc cactatccta actgcctcat gccccttcac 1539

tagttcatgc caagaacgtg actggaaagg catggcacct ataccttgat taatttccat 1599

taatggaaat ggttcgtcct gagtcatcta cgttccgagt caggctgtca ctcctacta  1658
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<210> 6
<211> 415
<212> PRT
<213> Homo sapiens

<400> 6

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Met Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln
  1               5                  10                  15

Lys Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr
             20                  25                  30

Leu Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val
         35                  40                  45

Ser Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val
     50                  55                  60

Leu Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr
 65                  70                  75                  80

Asn Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu
                 85                  90                  95

Leu Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe
            100                 105                 110

Leu Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr
        115                 120                 125

Val Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg
    130                 135                 140

Tyr Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg
145                 150                 155                 160
```

```
Arg Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu
                165                 170                 175

Phe Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe
            180                 185                 190

Pro Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys
        195                 200                 205

Pro Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe
    210                 215                 220

Tyr Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala
225                 230                 235                 240

Leu Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala
                245                 250                 255

Asn Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val
            260                 265                 270

Leu Val Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg
        275                 280                 285

Leu Phe Phe Ser Phe Val Glu Glu Trp Thr Glu Ser Leu Ala Ala Val
    290                 295                 300

Phe Asn Leu Val His Val Val Ser Gly Val Leu Phe Tyr Leu Ser Ser
305                 310                 315                 320

Ala Val Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala
                325                 330                 335

Ala Phe Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln
            340                 345                 350

His Asp Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu
        355                 360                 365

Cys His Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Leu Cys
    370                 375                 380

Gln Ser Ser Val His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu
385                 390                 395                 400

Gln Met Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
                405                 410                 415
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<210> 7
<211> 1658
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (64)..(1299)
<223> IGS4B short version

<400> 7
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat tttaatgtca 60

ggg atg gaa aaa ctt cag aat gct tcc tgg atc tac cag cag aaa cta 108
Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu
1 5 10 15

gaa gat cca ttc cag aaa cac ctg aac agc acc gag gag tat ctg gcc 156
Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala
20 25 30

ttc ctc tgc gga cct cgg cgc agc cac ttc ttc ctc ccc gtg tct gtg 204
Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val
35 40 45

gtg tat gtg cca att ttt gtg gtg ggg gtc att ggc aat gtc ctg gtg 252
Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val
50 55 60

tgc ctg gtg att ctg cag cac cag gct atg aag acg ccc acc aac tac 300
Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr
65 70 75

tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt gga 348
Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu Gly
80 85 90 95

atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg ttc 396
Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe
100 105 110

ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg tgc 444
Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys
115 120 125

ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac gtg 492
Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val
130 135 140

gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc cgg 540
Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg
145 150 155

gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc tcc 588
Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser
160 165 170 175

ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc aat 636
Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn
180 185 190

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ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc atg   684
Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met
            195                 200                 205

tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac ctc   732
Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu
        210                 215                 220

ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc aga   780
Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg
    225                 230                 235

cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat att   828
Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile
240                 245                 250                 255

caa aga ccc tgc aga aaa tca gtc aac aag atg ctg ttt gtc ttg gtc   876
Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu Val
                260                 265                 270

tta gtg ttt gct atc tgt tgg gcc ccg ttc cac att gac cga ctc ttc   924
Leu Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu Phe
            275                 280                 285

ttc agc ttt gtg gag gag tgg act gaa tcc ctg gct gct gtg ttc aac   972
Phe Ser Phe Val Glu Glu Trp Thr Glu Ser Leu Ala Ala Val Phe Asn
        290                 295                 300

ctc gtc cat gtg gtg tca ggt gtc tta ttc tac ctg agc tca gct gtc   1020
Leu Val His Val Val Ser Gly Val Leu Phe Tyr Leu Ser Ser Ala Val
    305                 310                 315

aac ccc att atc tat aac cta ctg tct cgc cgc ttc cag gca gca ttc   1068
Asn Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala Phe
320                 325                 330                 335

cag aat gtg atc tct tct ttc cac aaa cag tgg cac tcc cag cat gac   1116
Gln Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His Asp
                340                 345                 350

cca cag ttg cca cct gcc cag cgg aac atc ttc ctg aca gaa tgc cac   1164
Pro Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys His
            355                 360                 365

ttt gtg gag ctg acc gaa gat ata ggt ccc caa ttc cta tgt cag tca   1212
Phe Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Leu Cys Gln Ser
        370                 375                 380

tcc gtg cac aac tct cac ctc cca aca gcc ctc tct agt gaa cag atg   1260
Ser Val His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln Met
    385                 390                 395

tca aga aca aac tat caa agc ttc cac ttt aac aaa acc tgaattcttt    1309
Ser Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
400                 405                 410
```

cagagctgac tctcctctat gcctcaaaac ttcagagagg aacatcccat aatgtatgcc 1369

ttctcatatg aaattagaga ggtagaatgg ctcttacaac tcatgtaccc attgctagtt 1429

ttttttttt aataaacgtg aaaactgaga gttagatctg gtttcaaaac ccaagactgc 1489

ctgattttta gttatctttc cactatccta actgcctcat gccccttcac tagttcatgc 1549

caagaacgtg actggaaagg catggcacct ataccttgat taatttccat taatggaaat 1609

ggttcgtcct gagtcatcta cgttccgagt caggctgtca ctcctacta 1658

<210> 8
<211> 412
<212> PRT
<213> Homo sapiens

<400> 8

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Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu Glu
  1               5                  10                  15

Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala Phe
             20                  25                  30

Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val Val
         35                  40                  45

Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val Cys
     50                  55                  60

Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr Tyr
 65                  70                  75                  80

Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Gly Met
                 85                  90                  95

Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe Gly
            100                 105                 110

Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys Phe
        115                 120                 125

Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val Ala
    130                 135                 140

Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg Ala
145                 150                 155                 160

Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser Leu
                165                 170                 175

Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn Gly
            180                 185                 190

Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met Trp
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        195                 200                 205

Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu Leu
    210                 215                 220

Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg Leu
225                 230                 235                 240

Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile Gln
                245                 250                 255

Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Phe Val Leu Val Leu
            260                 265                 270

Val Phe Ala Ile Cys Trp Ala Pro Phe His Ile Asp Arg Leu Phe Phe
        275                 280                 285

Ser Phe Val Glu Glu Trp Thr Glu Ser Leu Ala Ala Val Phe Asn Leu
    290                 295                 300

Val His Val Val Ser Gly Val Leu Phe Tyr Leu Ser Ser Ala Val Asn
305                 310                 315                 320

Pro Ile Ile Tyr Asn Leu Leu Ser Arg Arg Phe Gln Ala Ala Phe Gln
                325                 330                 335

Asn Val Ile Ser Ser Phe His Lys Gln Trp His Ser Gln His Asp Pro
            340                 345                 350

Gln Leu Pro Pro Ala Gln Arg Asn Ile Phe Leu Thr Glu Cys His Phe
        355                 360                 365

Val Glu Leu Thr Glu Asp Ile Gly Pro Gln Phe Leu Cys Gln Ser Ser
    370                 375                 380

Val His Asn Ser His Leu Pro Thr Ala Leu Ser Ser Glu Gln Met Ser
385                 390                 395                 400

Arg Thr Asn Tyr Gln Ser Phe His Phe Asn Lys Thr
                405                 410
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<210> 9
<211> 1594
<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (55)..(942)
<223> IGS4A truncated DNA long version

<400> 9
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat ttta atg 57
Met

1

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tca ggg atg gaa aaa ctt cag aat gct tcc tgg atc tac cag cag aaa   105
Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys
                5                  10                  15

cta gaa gat cca ttc cag aaa cac ctg aac agc acc gag gag tat ctg   153
Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu
            20                  25                  30

gcc ttc ctc tgc gga cct cgg cgc agc cac ttc ttc ctc ccc gtg tct   201
Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser
        35                  40                  45

gtg gtg tat gtg cca att ttt gtg gtg ggg gtc att ggc aat gtc ctg   249
Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu
    50                  55                  60                  65

gtg tgc ctg gtg att ctg cag cac cag gct atg aag acg ccc acc aac   297
Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn
                70                  75                  80

tac tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt   345
Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu
            85                  90                  95

gga atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg   393
Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu
        100                 105                 110

ttc ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg   441
Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val
    115                 120                 125

tgc ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac   489
Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr
130                 135                 140                 145

gtg gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc   537
Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg
                150                 155                 160

cgg gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc   585
Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe
            165                 170                 175

tcc ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc   633
Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro
        180                 185                 190

aat ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc   681
Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro
    195                 200                 205

atg tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac   729
Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr
```

```
210                 215                 220                 225

ctc ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc   777
Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu
                230                 235                 240

aga cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat   825
Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn
            245                 250                 255

att caa aga ccc tgc aga aaa tca gtc aac aag atg ctg tct ttg tgg   873
Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Ser Leu Trp
        260                 265                 270

agg agt gga gtg aat ccc tgg ctg ctg tgt tca acc tcg tcc atg tgg   921
Arg Ser Gly Val Asn Pro Trp Leu Leu Cys Ser Thr Ser Ser Met Trp
    275                 280                 285

tgt cag gtg tct tct tct acc tgagctcagc tgtcaacccc attatctata      972
Cys Gln Val Ser Ser Ser Thr
290                 295

acctactgtc tcgccgcttc caggcagcat tccagaatgt gatctcttct ttccacaaac 1032

agtggcactc ccagcatgac ccacagttgc cacctgccca gcggaacatc ttcctgacag 1092

aatgccactt tgtggagctg accgaagata taggtcccca attcccatgt cagtcatcca 1152

tgcacaactc tcacctccca acagccctct ctagtgaaca gatgtcaaga acaaactatc 1212

aaagcttcca ctttaacaaa acctgaattc tttcagagct gactctcctc tatgcctcaa 1272

aacttcagag aggaacatcc cataatgtat gccttctcat atgatattag agaggtagaa 1332

tggctcttac aactcatgta cccattgcta gtttttttt tttaataaac gtgaaaactg 1392

agagttagat ctggtttcaa aacccaagac tgcctgattt ttagttatct ttccactatc 1452

ctaactgcct catgcccctt cactagttca tgccaagaac gtgactggaa aggcatggca 1512

cctatacctt gattaatttc cattaatgga aatggttcgt cctgagtcat ctacgttccg 1572

agtcaggctg tcactcctac ta                                          1594
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<210> 10
<211> 296
<212> PRT
<213> Homo sapiens

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<400> 10
Met Ser Gly Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln
  1               5                  10                  15

Lys Leu Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr
             20                  25                  30
```

```
Leu Ala Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val
         35                  40                  45

Ser Val Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val
     50                  55                  60

Leu Val Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr
 65                  70                  75                  80

Asn Tyr Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu
                 85                  90                  95

Leu Gly Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe
            100                 105                 110

Leu Phe Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr
        115                 120                 125

Val Cys Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg
    130                 135                 140

Tyr Val Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg
145                 150                 155                 160

Arg Arg Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu
                165                 170                 175

Phe Ser Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe
            180                 185                 190

Pro Asn Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys
        195                 200                 205

Pro Met Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe
    210                 215                 220

Tyr Leu Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala
225                 230                 235                 240

Leu Arg Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala
                245                 250                 255

Asn Ile Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Ser Leu
            260                 265                 270

Trp Arg Ser Gly Val Asn Pro Trp Leu Leu Cys Ser Thr Ser Ser Met
        275                 280                 285

Trp Cys Gln Val Ser Ser Ser Thr
    290                 295
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<210> 11
<211> 1594

<212> DNA
<213> Homo sapiens

<220>
<221> CDS
<222> (64)..(942)
<223> IGS4A truncated DNA short version

<400> 11
ggctcagctt gaaacagagc ctcgtaccag gggaggctca ggccttggat tttaatgtca 60

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ggg atg gaa aaa ctt cag aat gct tcc tgg atc tac cag cag aaa cta   108
    Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu
      1               5                  10                  15

gaa gat cca ttc cag aaa cac ctg aac agc acc gag gag tat ctg gcc   156
Glu Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala
                 20                  25                  30

ttc ctc tgc gga cct cgg cgc agc cac ttc ttc ctc ccc gtg tct gtg   204
Phe Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val
             35                  40                  45

gtg tat gtg cca att ttt gtg gtg ggg gtc att ggc aat gtc ctg gtg   252
Val Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val
         50                  55                  60

tgc ctg gtg att ctg cag cac cag gct atg aag acg ccc acc aac tac   300
Cys Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr
     65                  70                  75

tac ctc ttc agc ctg gcg gtc tct gac ctc ctg gtc ctg ctc ctt gga   348
Tyr Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu Gly
 80                  85                  90                  95

atg ccc ctg gag gtc tat gag atg tgg cgc aac tac cct ttc ttg ttc   396
Met Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe
                100                 105                 110

ggg ccc gtg ggc tgc tac ttc aag acg gcc ctc ttt gag acc gtg tgc   444
Gly Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys
            115                 120                 125

ttc gcc tcc atc ctc agc atc acc acc gtc agc gtg gag cgc tac gtg   492
Phe Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val
        130                 135                 140

gcc atc cta cac ccg ttc cgc gcc aaa ctg cag agc acc cgg cgc cgg   540
Ala Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg
    145                 150                 155

gcc ctc agg atc ctc ggc atc gtc tgg ggc ttc tcc gtg ctc ttc tcc   588
Ala Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser
160                 165                 170                 175

ctg ccc aac acc agc atc cat ggc atc aag ttc cac tac ttc ccc aat   636
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Leu Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn
                180                 185                 190

ggg tcc ctg gtc cca ggt tcg gcc acc tgt acg gtc atc aag ccc atg   684
Gly Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met
            195                 200                 205

tgg atc tac aat ttc atc atc cag gtc acc tcc ttc cta ttc tac ctc   732
Trp Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu
        210                 215                 220

ctc ccc atg act gtc atc agt gtc ctc tac tac ctc atg gca ctc aga   780
Leu Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg
    225                 230                 235

cta aag aaa gac aaa tct ctt gag gca gat gaa ggg aat gca aat att   828
Leu Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile
240                 245                 250                 255

caa aga ccc tgc aga aaa tca gtc aac aag atg ctg tct ttg tgg agg   876
Gln Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Ser Leu Trp Arg
                260                 265                 270

agt gga gtg aat ccc tgg ctg ctg tgt tca acc tcg tcc atg tgg tgt   924
Ser Gly Val Asn Pro Trp Leu Leu Cys Ser Thr Ser Ser Met Trp Cys
            275                 280                 285

cag gtg tct tct tct acc tgagctcagc tgtcaacccc attatctata         972
Gln Val Ser Ser Ser Thr
        290

acctactgtc tcgccgcttc caggcagcat tccagaatgt gatctcttct ttccacaaac 1032

agtggcactc ccagcatgac ccacagttgc cacctgccca gcggaacatc ttcctgacag 1092

aatgccactt tgtggagctg accgaagata taggtcccca attcccatgt cagtcatcca 1152

tgcacaactc tcacctccca acagccctct ctagtgaaca gatgtcaaga acaaactatc 1212

aaagcttcca ctttaacaaa acctgaattc tttcagagct gactctcctc tatgcctcaa 1272

aacttcagag aggaacatcc cataatgtat gccttctcat atgatattag agaggtagaa 1332

tggctcttac aactcatgta cccattgcta gtttttttttt tttaataaac gtgaaaactg 1392

agagttagat ctggtttcaa aacccaagac tgcctgattt ttagttatct ttccactatc 1452

ctaactgcct catgcccctt cactagttca tgccaagaac gtgactggaa aggcatggca 1512

cctatacctt gattaatttc cattaatgga aatggttcgt cctgagtcat ctacgttccg 1572

agtcaggctg tcactcctac ta                                          1594
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<210> 12
<211> 293

<212> PRT
<213> Homo sapiens

<400> 12

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Met Glu Lys Leu Gln Asn Ala Ser Trp Ile Tyr Gln Gln Lys Leu Glu
  1               5                  10                  15

Asp Pro Phe Gln Lys His Leu Asn Ser Thr Glu Glu Tyr Leu Ala Phe
             20                  25                  30

Leu Cys Gly Pro Arg Arg Ser His Phe Phe Leu Pro Val Ser Val Val
         35                  40                  45

Tyr Val Pro Ile Phe Val Val Gly Val Ile Gly Asn Val Leu Val Cys
     50                  55                  60

Leu Val Ile Leu Gln His Gln Ala Met Lys Thr Pro Thr Asn Tyr Tyr
 65                  70                  75                  80

Leu Phe Ser Leu Ala Val Ser Asp Leu Leu Val Leu Leu Leu Gly Met
                 85                  90                  95

Pro Leu Glu Val Tyr Glu Met Trp Arg Asn Tyr Pro Phe Leu Phe Gly
            100                 105                 110

Pro Val Gly Cys Tyr Phe Lys Thr Ala Leu Phe Glu Thr Val Cys Phe
        115                 120                 125

Ala Ser Ile Leu Ser Ile Thr Thr Val Ser Val Glu Arg Tyr Val Ala
    130                 135                 140

Ile Leu His Pro Phe Arg Ala Lys Leu Gln Ser Thr Arg Arg Arg Ala
145                 150                 155                 160

Leu Arg Ile Leu Gly Ile Val Trp Gly Phe Ser Val Leu Phe Ser Leu
                165                 170                 175

Pro Asn Thr Ser Ile His Gly Ile Lys Phe His Tyr Phe Pro Asn Gly
            180                 185                 190

Ser Leu Val Pro Gly Ser Ala Thr Cys Thr Val Ile Lys Pro Met Trp
        195                 200                 205

Ile Tyr Asn Phe Ile Ile Gln Val Thr Ser Phe Leu Phe Tyr Leu Leu
    210                 215                 220

Pro Met Thr Val Ile Ser Val Leu Tyr Tyr Leu Met Ala Leu Arg Leu
225                 230                 235                 240

Lys Lys Asp Lys Ser Leu Glu Ala Asp Glu Gly Asn Ala Asn Ile Gln
                245                 250                 255

Arg Pro Cys Arg Lys Ser Val Asn Lys Met Leu Ser Leu Trp Arg Ser
            260                 265                 270

Gly Val Asn Pro Trp Leu Leu Cys Ser Thr Ser Ser Met Trp Cys Gln
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275 280 285

Val Ser Ser Ser Thr
290

<210> 13
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerated primers

<220>
<221> variation
<222> (21)
<223> A,C,G or T

<220>
<221> variation
<222> (24)
<223> A, C, G or T

<400> 13
ctcatcttcg cggtgggcrc ngyngg 26

<210> 14
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerated primers

<220>
<221> variation
<222> (22)
<223> C or Inosine

<220>
<221> variation
<222> (25)
<223> A, C, G or T

<220>
<221> variation
<222> (28)
<223> A, C, G or T

<400> 14
ggccaggcag cgctccgcgc tnarncyngc d 31

<210> 15
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Degenerated primers

<400> 15
gaartartag ccrcgrcagc cw 22

<210> 16
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 16
ccatcctaat acgactcact atagggc 27

<210> 17
<211> 23
<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

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<210> 18
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<220>
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<400> 18
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<210> 19
<211> 29
<212> DNA
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<220>
<223> Description of Artificial Sequence: Primer

<400> 19
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<210> 21
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<210> 22
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<212> DNA
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<220>
<223> Description of Artificial Sequence: Degenerated primers

<400> 22
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<211> 27
<212> DNA
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<210> 24
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<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

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<210> 25
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
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<210> 26
<211> 30
<212> DNA
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<220>
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<210> 28
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<220>
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<210> 30
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

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<210> 31
<211> 22
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<220>
<223> Description of Artificial Sequence: Primer

<400> 31
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<210> 32
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 32
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<210> 33
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Primer

<400> 33
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<210> 34
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<220>
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<222> (1)
<223> Labeled with 6-carboxyfluorescein

<220>
<221> misc_binding
<222> (34)
<223> Labeled with
N,N,N',N'-tetramethyl-6-carboxyrhodamin

<400> 34
agatgtggcg caactaccct ttcttgttcg ggcc 34

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00202683.9	28 July 2000 (28.07.2000)	EP
60/222,047	31 July 2000 (31.07.2000)	US

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(72) Inventors; and

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(74) Agents: **VERHAGE, Marinus**; Octrooibureau Zoan B.V., C.J. Van Houtenlaan 36, NL-1381 CP Weesp et al. (NL).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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Published:
— *with international search report*

(88) Date of publication of the international search report: 11 October 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/25269 A3

(54) Title: HUMAN G-PROTEIN COUPLED RECEPTOR

(57) Abstract: The polynucleotides and polypeptides of the present invention relate to the G-protein coupled receptor family, referred to as IGS4-family. The invention also relates to inhibiting or activating the action of such polynucleotides and polypeptides, to a vector containing said polynucleotides, a host cell containing such vector and transgenic animals where the IGS4-gene is either overexpressed, misexpressed, underexpressed or suppressed (knock-out animals). The invention further relates to a method for screening compounds capable to act as an agonist or an antagonist of IGS4-family and the use of such IGS4 polypeptides, polynucleotides, agonists or antagonists. Preferred uses of the invention relate to disorders of the nervous system, disorders of the gastrointestinal system and/or of the cardiovascular system and/or of skeletal muscle and/or of the thyroid, and/or also to lung diseases, immunological diseases and disorders of the genitourinary system. The invention also relates to the identification of the cognate ligand of the IGS4 polypeptides the neuropeptides known as neuromedin U.

INTERNATIONAL SEARCH REPORT

Int ational Application No
PCT/EP 00/09584

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/705 C12N15/12 C07K16/28 C12Q1/68 A61K38/17
A01K67/027

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, PAJ, STRAND, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 4 August 1999 (1999-08-04), XP002131651 HINXTON, GB cited in the application AC = AC008571. HGT, Homo sapiens chromosome 5 clone CIT-HSPC_550M4, WORKING DRAFT SEQUENCE, 42 unordered pieces. From nt 204886-205679. abstract --- -/--	1-5,9, 17,18

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 March 2001	09/04/2001

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Mateo Rosell, A.M.

INTERNATIONAL SEARCH REPORT

In. .ational Application No
PCT/EP 00/09584

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 17 February 1996 (1996-02-17), XP002131652 HINXTON, GB cited in the application AC = N45474. EST, yy59b04.r1 Homo sapiens cDNA clone 277807 5'. abstract	1-5,9, 17,18
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 9 June 1996 (1996-06-09), XP002131653 HINXTON, GB cited in the application AC = W61169. EST, zd31a03.r1 Soares fetal heart NbHH19W Homo sapiens cDNA clone 342220 5'. abstract	1-5,9, 17,18
X	DATABASE EMBL NUCLEOTIDE AND PROTEIN SEQUENCES, 3 July 1995 (1995-07-03), XP002131654 HINXTON, GB cited in the application AC= H11359. EST, ym13d04.r1 Homo sapiens cDNA clone 47842 5' similar to contains L1 repetitive element abstract	1-5,9, 17,18
A	WO 90 01330 A (BENNETT TERENCE ;GARDINER SHEILA MARGARET (GB)) 22 February 1990 (1990-02-22) cited in the application abstract page 1, line 1 -page 5, line 3	6-8,19, 21, 28-30, 33-35, 38-40
Y	TAN C.P. ET AL.,: "Cloning and characterization of a human and murine T-cell orphan G-protein-coupled receptor similar to the growth hormone secretagogue and neurotensin receptors" GENOMICS, vol. 52(2), 1998, page 223-229 XP000879493 cited in the application Fig. 1 the whole document	1-5, 11-18

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INTERNATIONAL SEARCH REPORT

In. .ational Application No
PCT/EP 00/09584

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
Y	MCKEE K.K. ET AL.,: "Cloning and characterization of two human G protein-coupled receptor genes (GPR38 and GPR39) related to the growth hormone secretagogue and neurotensin receptors" GENOMICS, vol. 46(3), 1997, page 426-434 XP000889897 cited in the application Fig 1 the whole document	1-5, 11-18
A	VITA N ET AL: "CLONING AND EXPRESSION OF A COMPLEMENTARY DNA ENCODING A HIGH AFFINITY HUMAN NEUROTENSIN RECEPTOR" FEBS LETTERS,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 317, no. 1/02, 1 February 1993 (1993-02-01), page 139-142 XP002048144 ISSN: 0014-5793 cited in the application the whole document	1-5, 11-18
A	DATABASE WPI Section Ch, Week 199632 Derwent Publications Ltd., London, GB; Class B04, AN 1996-318958 XP002131656 & JP 08 143597 A (TAKEDA CHEM IND LTD), 4 June 1996 (1996-06-04) abstract	1-5, 11-18, 26-32
A	VITA N ET AL.,: "Neurotensin is an antagonist of the human neurotensin NT2 receptor expressed in Chinese hamster ovary cells" EUROPEAN JOURNAL OF PHARMACOLOGY, vol. 360, no. 2/3, 6 November 1998 (1998-11-06), pages 265-272, XP000879367 cited in the application the whole document	26-32
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P,X	WO 99 55732 A (AHMAD SULTAN ;CAO JACK (CA); DONNELL DAJAN O (CA); WALKER PHILIPPE) 4 November 1999 (1999-11-04) abstract page 2, line 1 -page 5, line 17; figure 2 SEQ.ID.N.1	1-5,9, 11-18, 23-26

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INTERNATIONAL SEARCH REPORT

Int. ational Application No
PCT/EP 00/09584

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 00 22131 A (ARENA PHARMACEUTICALS INC ;GORE MARTIN (US); LIAW CHEN W (US); LIN) 20 April 2000 (2000-04-20) abstract SEQ.ID.N.12 page 3, line 5-14; example 1 ---	1-5,9, 11-18, 23-26
P,X	HOWARD ANDREW D ET AL: "Identification of receptors for neuromedin U and its role in feeding." NATURE (LONDON), vol. 406, no. 6791, 2000, pages 70-74, XP000926239 ISSN: 0028-0836 the whole document ---	1-40
P,X	HOSOYA MASAKI ET AL: "Identification and functional characterization of a novel subtype of neuromedin U receptor." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 38, 22 September 2000 (2000-09-22), pages 29528-29532, XP002163224 ISSN: 0021-9258 the whole document ---	1-40
P,X	SZEKERES PHILIP G ET AL: "Neuromedin U is a potent agonist at the orphan G protein-coupled receptor FM3." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 27, 7 July 2000 (2000-07-07), pages 20247-20250, XP002163225 ISSN: 0021-9258 the whole document ---	6-8,19, 21, 28-30, 33-35, 38-40
P,X	FUJII RYO ET AL: "Identification of neuromedin U as the cognate ligand of the orphan G protein-coupled receptor FM-3." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 28, 14 July 2000 (2000-07-14), pages 21068-21074, XP002163226 ISSN: 0021-9258 the whole document ---	6-8,19, 21, 28-30, 33-35, 38-40

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/09584

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
T	RADDATZ RITA ET AL: "Identification and characterization of two neuromedin U receptors differentially expressed in peripheral tissues and the central nervous system." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 42, 20 October 2000 (2000-10-20), pages 32452-32459, XP002163227 ISSN: 0021-9258 the whole document ---	1-40
T	SHAN LIXIN ET AL: "Identification of a novel neuromedin U receptor subtype expressed in the central nervous system." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, no. 50, 15 December 2000 (2000-12-15), pages 39482-39486, XP002163228 ISSN: 0021-9258 the whole document -----	1-40

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 00 09584

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 23,24 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Although claim 25 is directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.2

Claims Nos.: 27,30,32

Claims 27,30 and 32, refer to an agonist and antagonist of polypeptide of claim 1 without giving a true technical charaterization. Moreover, no such compounds are defined in the application and in consequence, the scope of said claims is ambigous and vague, and their subject-matter is not sufficiently disclosed and supported according to Art. 5 and 6 PCT.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/EP 00/09584

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9001330	A	22-02-1990	NONE		
JP 8143597	A	04-06-1996	NONE		
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